Environmental Health Perspectives
Vol. 70, pp. 261-291, 1986

Radiation Effects in the Lung

by John E. Coggle,* Barrie E. Lambert,* and Steve R. Moores†

This article outlines the principles of radiobiology that can explain the time of onset, duration, and severity of the complex reactions of the lung to ionizing radiation. These reactions have been assayed biochemically, cell kinetically, physiologically, and pathologically. Clinical and experimental data are used to describe the acute and late reactions of the lung to both external and internal radiation including pneumonitis, fibrosis and carcinogenesis.

Acute radiation pneumonitis, which can be fatal, develops in both humans and animals within 6 months of exposure to doses ≥ 8 Gy of low LET radiation. It is divisible into a latent period lasting up to 4 weeks; an exudative phase (3–8 weeks) and with an acute pneumonitic phase between 2 and 6 months. The latter is an inflammatory reaction with intra-alveolar and septal edema accompanied by epithelial and endothelial desquamation. The critical role of type II pneumonocytes is discussed.

One favored hypothesis suggests that the primary response of the lung is an increase in microvascular permeability. The plasma proteins overwhelm the lymphatic and other drainage mechanisms and this elicits the secondary response of type II cell hyperplasia. This, in its turn, produces an excess of surfactant that ultimately causes the fall in compliance, abnormal gas exchange values, and even respiratory failure.

The inflammatory early reaction may progress to chronic fibrosis. There is much evidence to suggest that pneumonitis is an epithelial reaction and some evidence to suggest that this early damage may not be predictive of late fibrosis. However, despite detailed work on collagen metabolism, the pathogenesis of radiation fibrosis remains unknown.

The data on radiation-induced pulmonary cancer, both in man and experimental animals from both external and internal irradiation following the inhalation of both soluble and insoluble alpha and beta emitting radionuclides are reviewed. Emphasis is placed on the data showing that alpha emitters are at least an order of magnitude more hazardous than beta/gamma radiation and on recent data showing that the more homogeneous the irradiation of the lung, the greater is the carcinogenic hazard which contradicts the so-called "hot particle" theory.

Introduction

The reaction of the lung to radiation is an involved one, since it is a complex organ. Over 40 types of cell make up the lung, and most of them would be considered relatively radioresistant (*1*). However, since the lung as a whole has little regenerative capacity, it cannot tolerate large doses of radiation and its radiosensitivity is the major limiting factor in radiotherapy of the chest (*2,3*). The critical injuries that eventually lead to impaired ventilation and diffusion capacity are related to the total dose, its fractionation, and to the volume of the lung irradiated. It is well established that the absorption of ionizing radiation causes immediate biochemical, subcellular, and cellular damage, while its morphological expression in terms of gross tissue injury and organ dysfunction are often considerably delayed. The latent period between the exposure and the expression of damage is critically dependent on how efficiently the normal cells can repopulate the tissue (*4*).

*Department of Radiation Biology, Medical College of St. Bartholomew's Hospital, Charterhouse Square, London, EC1M 6BQ, U.K.

†Inhalation Toxicology Group, Environmental and Medical Sciences Division, AERE Harwell, Oxon. OX11 ORA, U.K.

Radiation damage to the lung can be described at all levels of organization from the molecular through to the organ level. The response of the lung has been assayed biochemically (*5–8*), cell kinetically (*9–11*), histologically (*12–14*), physiologically (*15–17*), and pathologically, using lung death as an endpoint (*18,19*).

In this review we shall first outline some of the general principles of radiobiology that can explain time of onset of appearance and the extent and the severity of radiation responses in organized tissues. Then we shall draw upon both human and experimental animal data to describe the significant early and late reactions of the lung to external and internal radiation, including pneumonitis, fibrosis, and carcinogenesis.

General Aspects of Radiation Effects in Organized Tissues

The response of mammalian tissues to radiation is the integral of cell death and damage together with its repair capacity.

The extent of the damage suffered by a tissue such as the lung is a complex function of physical and bio-

logical factors. The physical factors include the size of the radiation dose, its quality and whether the exposure is single, fractionated or protracted. The biological factors include the radiosensitivity of the different cells of the tissue, their population kinetics, their state of oxygenation, the differential sensitivity of the phases of the mitotic cycle and, perhaps most important, the presence or absence of repair and repopulation processes.

The ability of ionizing radiation to cause the mitotic death of cells is fundamental to an understanding of tissue radiobiology (*20*). It has been known since 1906 that the radiosensitivity of a tissue is directly related to its mitotic activity and inversely proportional to the degree of differentiation of its cells (*21*). Consequently, the liver, kidney, muscle, bone, lung, and connective tissues are often classified as radioresistant, while the rapidly proliferating cells of the bone marrow, the germinal cells of the testis, and the epithelial cells of the skin, intestine, and stomach are all classified as radiosensitive (*22,23*).

At very high doses (tens of Gray*), radiation can cause the cessation of metabolism and cellular disintegration—a type of death known as interphase death. However, much lower doses (2–3 Gy) will sterilize two-thirds of a population of cells by inhibiting their ability to divide (*24*). Some of the irradiated cells will degenerate and die at the first post-irradiation mitosis, while others may successfully undergo one or two divisions before dying at the third or fourth post-irradiation mitosis. There is evidence to link such reproductive death with DNA (*25*). It is a fundamental tenet of radiobiology that the response of a tissue is contingent upon the level of cell killing.

The graphical presentation of the fraction of surviving cells plotted logarithmically against the radiation dose plotted linearly is known as a survival curve. The exponential slope of such a survival curve is described by a value known as D_0, which is the dose to reduce the surviving fraction of cells to 37% of any initial value. Survival curves have been produced *in vitro* and *in vivo* for numerous cell lines, and D_0 values for acute doses of low LET radiation (X-rays, gamma-rays and electrons) are generally between 1 and 3 Gy.† For high LET radiation (alpha particles and neutrons) D_0 values tend to be somewhat smaller. After low doses (1–2 Gy) of low LET radiation there is a finite chance that some cells will be killed but most suffer what is called "sublethal" damage (SLD), and in radiotherapy the use of fractionated dose regimes is designed to allow the normal tissues in the therapy beam time to repair at least some of this sublethal damage in the intervals between successive dose fractions. It will be obvious that a series of smaller doses is even less effective than larger fractions, and continuous, low dose rate, chronic radiation, which can be regarded as ultrafractionation, is the least effective way of damaging a tissue. As the dose rate gets lower and lower, the survival curves get less steep until the rate of repair balances the rate of SLD induction and for many systems with low LET radiation this limiting slope occurs at about 0.1 Gy/hr.

Besides these important time–dose factors associated with SLD repair there are many other factors that can modulate the effectiveness of a given radiation exposure. These include the repopulation kinetics of surviving cells, the repair of potentially lethal damage, and various slow repair processes (*26–31*).

The most important of the factors determining the extent of injury in a tissue is its ability to repopulate after radiation damage (*4,31*). This repopulation must involve both the dividing stem cells and the nondividing functionally mature cells. The former will begin to die when they attempt their first or second post-irradiation divisions. While the nondividing differentiated cells, relatively unaffected by radiation, will continue to function and to die at their normal rate, they will not be so efficiently replaced because of the damage to the stem cell compartment. This time course of the repair is closely related to the efficiency of the population kinetics. Injury will not become apparent until the number of functional cells falls below a critical level. The extent of the damage in a tissue will of course be related to dose, which will determine the severity and duration of the cellular depletion. In contrast, the time of onset of damage is less dependent on the size of the dose and more dependent on the cell kinetics of the tissue. For example, in rapidly dividing tissues, e.g., the skin, gastrointestinal mucosa, and testis, cell death and cell depletion will occur earlier than in slowly dividing tissues such as kidney and lung (*32*). In the latter the damage may be delayed for months, although the onset of damage, when it occurs, may be quite acute; these so-called "delayed acute reactions" have been seen in the kidneys and lungs. For protracted irradiation the dose rate at which the rate of cell production balances the rate of cell death varies enormously from tissue to tissue (*33*). In the testis, deleterious effects are detectable at dose rates as low as 0.01 Gy per day (*34,35*), while 0.5 Gy and 4 Gy per day, respectively, are reported as the critical daily dose rates for the erythropoietic system of rodents and for the rat intestine (*36*). In the lung and other slowly proliferating systems there are virtually no data on the effects of low-level chronic irradiation.

Radiation causes acute effects (within days or weeks) in rapidly proliferating tissues and delayed effects (months or years) in slowly or nonproliferating tissues. There is general agreement that acute effects are primarily due to a disturbance of cell kinetics. In contrast, there is much debate about the pathogenesis of the late effects seen in slowly dividing tissues. One school of thought suggests that late effects, excluding cancer induction, are due primarily to vascular damage (*37*). It is held that damage to small vessels eventually leads to a degeneration of cells of a tissue and to the generalized late fibrosis of its connective tissues. The other school

*Grays are the unit of absorbed dose: the amount of enery imparted to unit mass of matter, such as tissue. 1 gray (Gy) = 1 joule per kilogram.

†Linear Energy Transfer (LET) is a measure of the density of ionization events along the track of the radiation in a medium such as tissue.

of thought suggests that the wide diversity of late damage seen in different tissues is best explained in terms of the slowly proliferating parenchyma of such tissues (*32*). This hypothesis suggests that, just as acute effects occur early in rapidly dividing tissues, so the late effects occur late in slowly dividing tissues because the expression of the damage at mitosis has to await the gradual appearance of such divisions. Of course, late effects may be due to a combination of changes in the connective tissues, the parenchyma, and the vascular elements.

In comparison with many tissues, e.g., the skin, bone marrow, and gastrointestinal tract, our knowledge of the radiobiology of the lung is rudimentary. However, many of the precepts just described form a useful framework within which to view what is known of the radiosensitivity and radioresponsiveness of the lung.

Acute Effects of Radiation in the Lung

Introduction

The significant acute and late reactions of the lung to radiation have been described in some excellent reviews (*3,14,38–40*). The lung's response, both clinically and in experimental animals, is similar and can be divided into two syndromes that are not necessarily related. They are (a) radiation pneumonitis, which develops within 6 months after exposure to doses $\geq$ 8 Gy of X – or gamma-rays and (b) radiation fibrosis, which is a delayed or late reaction that develops from about 6 months to years after exposure.

Radiation pneumonitis itself can be divided into a number of phases, which although they merge and overlap one another are nevertheless distinct. These are: (i) a latent period, which lasts up to 4 weeks after moderate doses (~10–15 Gy) and is characterized by the virtual absence of gross histological damage. (ii) An exudative period, 3–8 weeks post-irradiation, which is characterized by protein-rich deposits in the air spaces, with increasing epithelial and endothelial cell damage. At low doses (8–10 Gy) this phase may be nonexistent. (iii) Acute pneumonitis, which occurs 2–6 months after exposure, involves edema of the air spaces and the alveolar septa. Desquamative changes occur in epithelial and endothelial cells, with increasing numbers of mononuclear, inflammatory cells in the septa and air spaces. (iv) The late or chronic phase occurs from 6–12 months onwards and involves repair proliferation of septal and alveolar cells that leads to subtle reconstructional changes in septal, vascular, and connective tissue elements. This finally merges into the progressive consolidative increase in interstitial fibrosis and capillary sclerosis that characterizes late radiation fibrosis.

Radiation pneumonitis is generally regarded as an inflammatory reaction that progresses to a chronic fibrotic reaction. Deaths can occur in both the acute and the late phase.

There is not yet a generally accepted theory on the pathogenesis of radiation pneumonitis, although there are several hypotheses as to what may be the primary lesion. It has often been suggested that the damage is principally vascular with a sloughing of dead and dying endothelial cells, causing capillary leakage both interstitially and onto the alveolar surface (*2,8,12,41,42*). Other work highlights damage to type II cells (*14*), with the death and dysfunction of such epithelial cells causing serious alteration in the levels of surfactant phospholipids (*6,43,44*). Yet other work suggests the primary damage is the necrosis and sloughing of type I cells, which leaves denuded basement membranes and alveolar debris (*12,39,45*). It is further suggested that it is the subsequent hyperplasia and differentiation of type II cells that repair the alveolar epithelial layer and it is the efficiency of such repair that determines the extent of the final injury (*39*). Finally, there are minor references implicating a critical role for the lymphocytes (*46*), the immune system (*47*), and microbial infection (*13,48*).

In the following paragraphs we shall enlarge on some of these ideas but it must be said that our present knowledge is insufficient to allow a definite choice to be made between these hypotheses.

The Latent Period of Pneumonitis

In this phase, at about one month post-irradiation, the lungs do not exhibit the typical inflammatory reactions of acute pneumonitis. There is no evidence from light microscopy of damage at less than 3 weeks after 10–20 Gy of X-irradiation. Nevertheless there are increasing numbers of studies highlighting important biochemical, ultrastructural, and cellular changes that precede the gross histological and physiological evidence of pneumonitis. Such early changes may provide useful clues to the later events in radiation pneumonitis.

Maisin (*12,49*) was one of the first to use electron microscopy to detail the very early post-irradiation effects. After a dose of 20 Gy to the rat lung, he observed focal lesions involving ultrastructural changes in cell types, within 3 hr of irradiation. The changes included disruption of plasma membranes, with widening and invagination of the perinuclear space. Capillary permeability was increased, probably due to membrane changes in the endothelial cells. These changes continued, and by 6 hr, cellular ultrastructure was markedly altered: mitochondria in all cell types were affected with dilation of the matrix, disruption of the cristae, and the presence of myelin-like fibers. The lamellar bodies of type II cells were enlarged and irregular—although not increased in number—and both type I and type II cells had developed autophagic vacuoles. Hypertrophy and vacuolation of the cytoplasm of endothelial cells caused obstruction and swelling of the capillaries which persisted through the first week after irradiation.

Most of these effects have been confirmed by other authors, although there is still a dearth of such data and no consensus as to the exact pattern of cause and effect of the pathogenesis of these early effects. Phillips (*2,18*) reported that the earliest damage in the rat lung was to the microvasculature, with endothelial injury prom-

inent 24 hr after 20 Gy. All the damage was ascribed to the capillaries, and the authors stated that there was "no alteration in the epithelium or basement membrane" (*18*). Adamson and his colleagues (*45*) described similar endothelial changes in rat lung within 2 days of 11 Gy and 5 days of 6.5 Gy, with the degeneration of the endothelium leading to distension and blockage of the capillary lumen. Finally, in the dog following one-lung irradiation with X-rays, it has been reported that the initial site of damage was to the capillaries and their endothelium (*50*). As early as 14 days after irradiation, there was luminar dilation and congestion, with increased permeability causing interstitial edema. The changes were minimal in the 2–8 Gy group, but most significant within the highest dose one (24–32 Gy).

In contrast, "no widespread endothelial injury" was found in an ultrastructural study of the effect of single and fractionated doses of 10, 20, and 30 Gy X-rays, either as single doses or equal fractionated exposures for 5 consecutive days at times from 1 hr to 1 month (*51*). In addition, pinocytic activity was essentially unaltered by the radiation. They noted an increase in the microvilli projecting into the capillary lumen, and they support the observations of Maisin (*12*) that such projections might impede capillary flow and encourage thrombocytosis. However, they stressed that at the ultrastructural level the primary and most marked changes involved the type I and type II pneumocytes, with an associated increased edema, fibrin deposition, and histocyte invasion occurring within 24 hr. There was a marked decrease in the number of lamellated bodies in type II cells at 24 hr with type II hyperplasia and an increase in such bodies 7 days later. The vacuolation, mitochondrial swelling, loss of internal membranes, and distortion of the endoplasmic reticulum of type II cells and their sloughing into the alveolar lumina occurred 3–4 weeks post-irradiation. Curiously, both type II autolysis and other cellular damage was more marked in the lungs treated with the fractionated doses. Occasionally, type I cells showed signs of progressive degeneration with a loss of organelle integrity as early as 24 hr. Although the alveolar wall was edematous and had areas of thickened basement membrane, no areas of denuded membranes were observed.

In contrast, Adamson and co-workers (*45*) described focal swelling, necrosis, and sloughing of type I cells to leave a denuded basement membrane. The effect was maximal at 10 days after 11 Gy whole body, 14 days after 6.5 Gy whole body or 30 Gy to the hemithroax. There was, however, no damage to the type II cells "nor was there proliferation of type II cells such as occurs after exposure to oxygen and nitrogen dioxide."

Goldenberg et al. (*52*) found early changes in type II cells in rats, but no effect on type I or endothelial cells, while in hamsters given a high dose of 100 Gy of X-rays to the lungs, Madrazo et al. (*47*) showed progressive ultrastructural changes in all alveolar cell types in the hamster lung within 14 days. The regenerative hyperplastic response of the type II cells was reported to be very efficient and no denudation of the basement membranes was seen.

Early ultrastructural changes in experimental animals have been reported after high doses of internal alpha and beta emitters (*53*). It is clear that there are reports in the literature of ultrastructural focal lesions occurring in most species within a few days in all types of epithelial and endothelial cells, interstitial cells, plasma and basement membranes and even in the alveolar macrophages (*12,54*). It is also clear that there is little agreement in the literature as to which lesions are the most crucial in this early latent period. There is a need for further ultrastructural studies to clarify the early sequence of events. However, if such studies are to be of value in predicting the possible mechanisms of acute pneumonitis, they should be combined with biochemical and functional studies. They should also involve a wider range of doses than has usually been the practice in electron microscopy studies.

The Exudative Phase of Pneumonitis

One of the earliest, although not the most consistently reported response both clinically and experimentally, is the appearance of fibrin-rich serum proteins on the alveolar surfaces (*14*). It is usually seen during the first 30 days following radiotherapy and in some patients is associated with hyaline membrane formation (*3,40*). Hyaline membranes are rarely reported in experimental animals (*18,19,51,55*). Ahier et al. (*56*) reported a consistent dose-dependent increase in serum protein levels in alveolar lavage fluid in mice given thoracic X-ray doses. At $\leq$ 10 Gy this proteinosis was transient, peaked at ~4 weeks and was resolved by 6 weeks post-exposure. After ~15 Gy there was a three- to fourfold increase in protein levels by 4 weeks and this persisted into the phase of pneumonitis, some 4 months post-irradiation. By this time the protein levels in lavage fluid could be as high as twenty times those of the controls.

Such hematogeneous leakage easily and rapidly interferes with lung function by reducing the volume of the air space and by increasing the barrier to gaseous diffusion. In rats, following inhalation of $^{238}PuO_2$ and $^{239}PuO_2$ severe edema and alveolar flooding with proteinaceous fluids occurred within 6–7 days of exposure (*56*).

In contrast, Gross (*8*) in mice, given 30 Gy as two fractions separated by 48 hr, found no increase in the protein levels in alveolar lavage fluids up to 8 weeks post-treatment, but at 16 weeks the amount was 4–5 times normal. Gross emphasizes this "period of 3–5 months before the endothelium and epithelium becomes leaky." Nevertheless, the clinical and experimental reports of early proteinosis are consistent with the early ultrastructural changes in the fine vasculature and the alveolar epithelium and with the early variations in capillary permeability and perfusion that have been reported. For example, Teates (*58*) reported a transiently increased, pulmonary diffusion capacity for carbon mon-

oxide in rats within 1 week of 30 Gy of X-rays, which would indicate increased capillary perfusion. Freedman et al. (*59*) observed increased pulmonary capillary perfusion in rabbits during the first week after single doses of 15–30 Gy of X-rays to the right lung. There are two reports of transitory reductions in perfusion occurring within 1–2 hr of doses between 30–60 Gy of X-rays in rabbits and in both studies the perfusion returned to normal within 24 hr (*59,60*). Travis and co-workers (*61*) found an increase in rats, 2, 4, and 8 weeks after 5, 20, and 40 Gy of X-rays, and they state that such effects preceded any histological evidence of lesions. Henderson et al. (*62*) showed that in dogs that had inhaled beta-emitting isotopes—yttrium- and cerium-labeled insoluble particles—an increased leakage of protein into the alveolar space occurred less than 1 month after accumulated doses of 60–100 Gy beta irradiation. Studies in man also show that a reduction in perfusion precedes the signs and symptoms of pneumonitis and occurs as early as 3–4 weeks after thoracic irradiation (*17,63–65*).

The evidence for early changes in the permeability of the interstitium seems equivocal. The major changes in the vascular and mechanical properties of the lung occur in the main period of morbidity and mortality, 3–6 months post-irradiation.

Acute Radiation Pneumonitis

Introduction

The main histological lesions that characterize the acute phase of pneumonitis in both humans and experimental animals are inflammation and edema of the interstitium and air spaces. Excess numbers of mononuclear inflammatory cells, including foamy macrophages, infiltrate the area. The air spaces usually contain excess protein and fibrin-rich exudates. Besides these most obvious responses, there are reports of lesions in virtually all lung structures (*39*). As Gross (*39*) remarks, "there are no specific lesions that entirely characterise radiation pneumonitis." Table 1 lists the principal histopathological abnormalities of pneumonitis. Not only are these responses seen in animals receiving external X- and gamma-radiation, but the same sequence and timing of cell and tissue lesions have been described in rats, mice, hamsters, dogs, and rabbits whose lungs have been irradiated by instillation or inhalation of alpha- and beta-emitting radionclides (*66–69*) and reviewed in ICRP 31 (*53*). Clinical data are less systematic, but it is well established that acute doses $\geq$ 7–8 Gy X-irradiation produce similar widespread damage in all elements of the human lung (*38*). Despite much work, the pathogenesis of pneumonitis is still poorly understood. Damage to the capillary endothelial cells has been cited as the major target (*2,70,71*), while there is increasing support for the primacy of type II cell damage as the cause of acute radiation pneumonitis (*6,14,38,39,51,72*). There is also recent evidence suggesting a dissociation between the two distinct types of lung damage—pneumonitis occurring at 3–6 months and fibrosis at $\geq$ 6 months after radiation (*73,74*).

Post-Irradiation Cell Kinetics in the Lung

Radiation preferentially kills proliferating cells, and so the expression and appearance of damage in the lung will be a function of the turnover rate of the cells that comprise the lung. However, in comparison with the skin, gut, testis, bone marrow, and many other tissues, very little attention has been paid to the post-irradiation cell kinetics of the lung. There are obvious reasons that militate against good cell kinetics in the lung. First, there is the inherent difficulty of cell recognition under light microscopy and autoradiography. Secondly, the low cell density and low turnover rate of lung cells necessitate scoring many sections for statistically significant numbers of DNA-labeled cells. Finally, among the 40 or so cell types, there is no dominant one that might allow one to use any overall parameter as representative of the kinetics of the lung as a whole.

Despite these limitations, some data exist on the two cell populations that show the most active proliferation: type II cells and alveolar macrophages. These cells are also likely to be the most radioresponsive.

Type II Pneumonocytes. The response of type II cells is of great interest not only because they play a role in pneumonitis but as they are also likely target cells for radiation-induced adenomas in rodents (*10,75*). Furthermore, they are the source of surfactant and act as the stem cell population for type I cells (*76–78*), which rarely if ever divide (*79–82*). Despite the importance of these cells, only one or two studies of post-irradiation response have been published. For example, the DNA labeling index of type II epithelial cells of mice given thoracic X-ray doses of 2, 5, and 10 Gy showed an initial 7-day depression followed by a compensatory overshoot, so that at 3 months post-irradiation the labeling index (LI) was five times the control mice (*83*). The large errors inherent in a system with a LI as low as that of type II cells (~0.4%) have been noted by others. Coultas and co-workers (*9*) showed that the proliferative response in type II cells of mice, after either 7.7 Gy neutrons or 10 Gy of X-rays, was identical; that is, reduced for the first 6 days and then gradually rising above controls, 3 to 5 weeks post-irradiation. It could not therefore be the explanation for the difference in response to the two radiations i.e., "slow repair" after X-rays but not after neutrons. Furthermore, the repopulation of type II cells only occurred at a time when the slow repair was essentially complete.

These two studies highlight the important point that radiation produces quantitatively and qualitatively, different lung cell kinetics from most other toxic agents. For example, ozone (*79*), nitrogen dioxide (*78*), bleomycin (*77*) and urethane (*84*) all produce immediate and severe damage to type I and type II cells with concomitant prompt repair, involving striking increases in type II cell proliferation. In contrast, the radiation response is decidedly low key and chronic, which presumably reflects the low turnover rate and consequently delayed genetic cell death of the alveolar epithelial cells. To circumvent the disadvantages of slow turnover, Meyer et al. (*10,75*) have developed a method to assess the

Table 1. Histopathological changes in the lung after radiation.[a]

Site	Immediate and early (0–2 months)	Intermediate (2–9 months)	Late (9 months+)
Capillaries	2 hr: Endothelial changes leading to increased permeability 2 day: Endothelial separation from basement membrane. Sloughing of cells giving obstruction of capillary lumen	Abnormalities Widespread obstruction due to platelets, collagen and fibrin Reduced capillary permeability	Loss of many capillaries Regeneration of new capillaries Reduced permeability
Type I cells	Degenerative changes and sloughing or Normal Decreased numbers	Further decrease	
Type II cells	Early degenerative changes becoming more marked with time or Normal	Large increase in size and number Abnormal appearance	Return to normal size and number
Basement membrane	Swollen, becoming irregular	Folded and thickened	Folded and thickened
Interstitial space	Edema and debris. Increase in number of inflammatory cells Slight increase in coarse connective tissue	Infiltration of mononuclear, mast and inflammatory cells Increase in connective tissue	Few inflammatory cells Large increase in collagen
Alveolar space	Hemorrhagic Filled with fibrin and cell debris Increase in number of alveolar macrophages	Alveolar space becomes smaller	Alveolar space small or absent Architecture destroyed

[a] Modified from Gross (*38*), with permission.

post-irradiation proliferative response of type II cells after stimulating their division with the antioxidant, butylated hydroxytoluene (BHT), which selectively kills type I cells. The D_0 values for the proliferating fraction (PF) of type II cells were 1.2 Gy and 0.6 Gy for X-rays and fission neutrons, respectively. These values were obtained when the BHT stimulus was given immediately after radiation and measured by the inhibition of uptake of labeled DNA precursors. The D_0 (PF) for X-rays increased from 1.2 Gy to 3.6 Gy at day 2 and to 5.8 Gy at day 14 after BHT. After neutrons, on the other hand, the D_0 (PF) was 0.6 Gy at both day 0 and day 2 after BHT stimulation, but increased to 3.45 Gy at day 14. The evidence from split dose studies shows that type II cells are capable of both short-term (1–2 days) and longer term (several weeks) recovery, which might be the cellular basis of the phenomenon of slow repair (*85*).

Alveolar Macrophages. Radiation biologists also have a particular interest in the post-irradiation kinetics of alveolar macrophages (AM) because they play a major role in the clearance of radioactive particles and thus modulate the final dose to the lung tissue. Much of the early work dealt with the immunological role of AMs and is of limited value because it involved single doses and single assay times (*86–89*). These and other studies on the effects of external X-, gamma-, or neutron-irradiation on AM function have resulted in conflicting observations. Thus, suppression of AM function by radiation has been reported (*90,91*) as has their resistance to radiation (*92–95*).

In a recent study, the rate of pulmonary clearance of inhaled *Staphylococcus aureus* in mice was determined at intervals after inhalation exposure to either the short-lived beta-emitter, $^{144}CeO_2$ or the long-lived alpha-emitter, $^{239}PuO_2$ (*96*). Both radionuclides are relatively insoluble and both produced a reduction in clearance of the bacteria. For example, 4.7 μCi of ^{144}Ce suppressed clearance for up to 12 weeks post-inhalation, while 29 nCi of ^{239}Pu suppressed clearance for up to 26 weeks. The suppressed clearance did not correlate with the radiation-induced histopathological changes, but did correlate with the dose rate to the lungs at the time of bacterial exposure. The study did not quantitate the response of AM, but the authors suggest that "direct radiation injury to the AM population is the likely cause of reduced pulmonary clearance." This suggestion receives substantial support from three independent studies using endobronchial lavage to measure the fall and subsequent recovery of AM numbers after a range of doses from both external X-irradiation (*11,97*) and internal alpha-emitters (*98*).

Figure 1 shows a typical pattern of response in X-irradiated mice. The absolute number of AM recovered by lavage from controls in this experiment was $\sim 10^6$ per mouse, which is some 40% of the total AM pool.

Figure 2 taken from the data of Moores and co-workers (*98*) shows the changes in total number of AM in the mouse lung after inhalation of $^{239}PuO_2$. The alpha-emitting particles produced both an acute depression in AM numbers and, at high lung burdens, a sustained chronic depletion.

The depletion in all these studies was dose dependent and sensitive to fractionation (*38*), consistent with a radiosensitive, proliferating pool of intrapulmonary macrophage or macrophage precursor cells. Recent cell kinetic studies, some using radiation, lends support to this idea of subpopulations of pulmonary macrophages

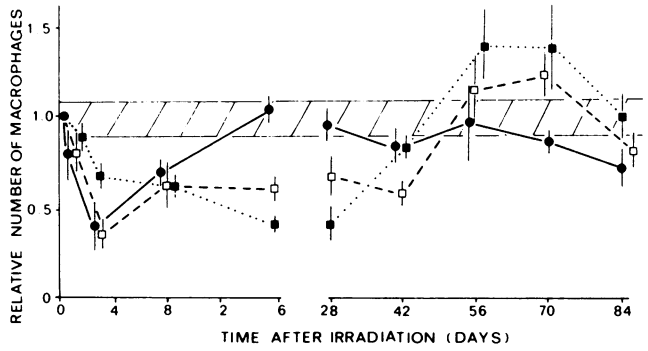

FIGURE 1. Relative number of alveolar macrophages recovered by bronchoalveolar lavage from mice after thoracic X-irradiation: (●)2 Gy; (□) 5 Gy; (■) 10 Gy. (*11*).

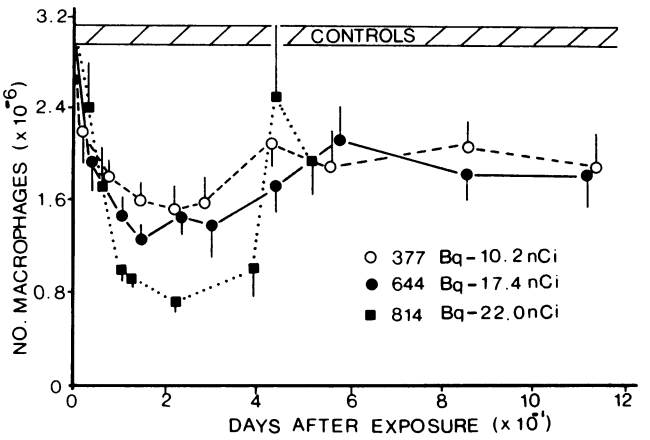

FIGURE 2. Total number of alveolar macrophages in the lungs of mice, after inhalation of $^{239}PuO_2$, alpha-emitting particles. (*98*).

that have proliferative capacity (*99–102*). It is probable that these dividing macrophages are radiosensitive. The survival curves for both mouse and hamster alveolar macrophage colony forming cells are reported to be characterized by a D_0 of about 2 Gy for ^{137}Cs gamma-rays (*103*).

It has been suggested that macrophages play only a minor role in radiation pneumonitis because the perturbations in the AM population precede pneumonitis by several weeks and pulmonary defense mechanisms in general seem to have little to do with pneumonitis (*39*). On the other hand, AM are seen in excess numbers often as "foamy cells" filling alveolar spaces during acute pneumonitis and before dismissing them one really needs systematic and long-term data on the effects of radiation on the AM population size at 3–9 months post-irradiation.

The importance that several authors attach to the role of vascular lesions in the development of acute pulmonary damage has been noted and we shall enlarge on this later in relation to the delayed effects. It is suggested that the primary lesion is endothelial cell death that occurs when the radiation sterilized cells go into mitosis. However, since the turnover time of endothelial cells is long—months to years (*37*)—significant vascular damage will occur very late after radiation. There seems to be no significant published data on the normal or post-irradiation kinetics of endothelial cells in the lung.

Experimental Pulmonary Lethality

The most common endpoint used to measure the death of animals from pneumonitis is the $LD_{50/180}$, which is the dose required to kill 50% of animals between 80 and 180 days after treatment (*18*). This $LD_{50/180}$ value varies with species and strain but is generally between 10 and 14 Gy for single acute doses of X-rays.

It can be argued that type II cells, macrophages and endothelial cells are all functionally similar in different strains and species, the inference being that variations in $LD_{50/180}$ values for pneumonitis cannot be due to effects on these cells. However, the more powerful counter argument would be that while it is probably true that the cell types mentioned are indeed structurally and functionally identical, there are likely to be subtle though important species/strain variations in the radiosensitivity and in the post-irradiation cell kinetics that could easily account for the relatively small variations in $LD_{50/180}$ doses. This argument receives strong support by analogy with radiation induced hemopoietic death where strain/species variations can be quite accurately correlated with the critical pluripotent stem cell compartment, its size, radiosensitivity and post-irradiation recovery kinetics.

Figure 3 shows typical survival curves for the percentage of mice surviving to 180 days after thoracic irradiation with single doses, or two equal doses separated by 24 hr, of 250 kVp X-rays or fast neutrons (*71*). It shows the reduced effectiveness of fractionation and taken together with data in Figure 4, shows that there

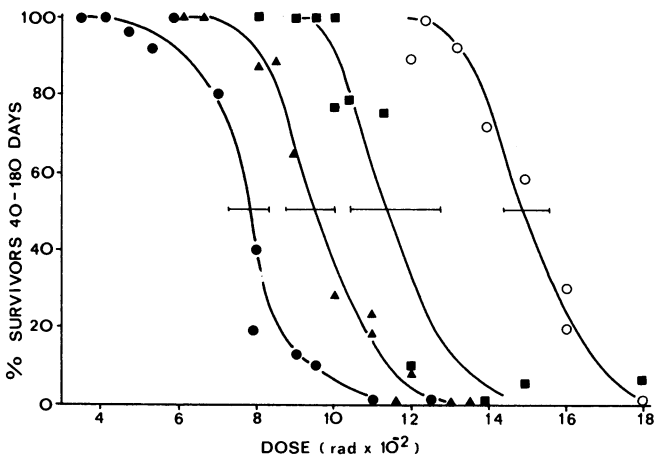

FIGURE 3. Percentage of mice surviving at 180 days after irradiation of the thorax with single doses or two equal doses of 250 kVp X-rays or fast neutrons, separated by 24 hr: (●) single neutron doses: (▲) two fractions neutrons; (■) single X-ray doses; (○) two fractions X-rays. From S. B. Field and S. Hornsey (*71*), courtesy of the authors and Pergamon Press.

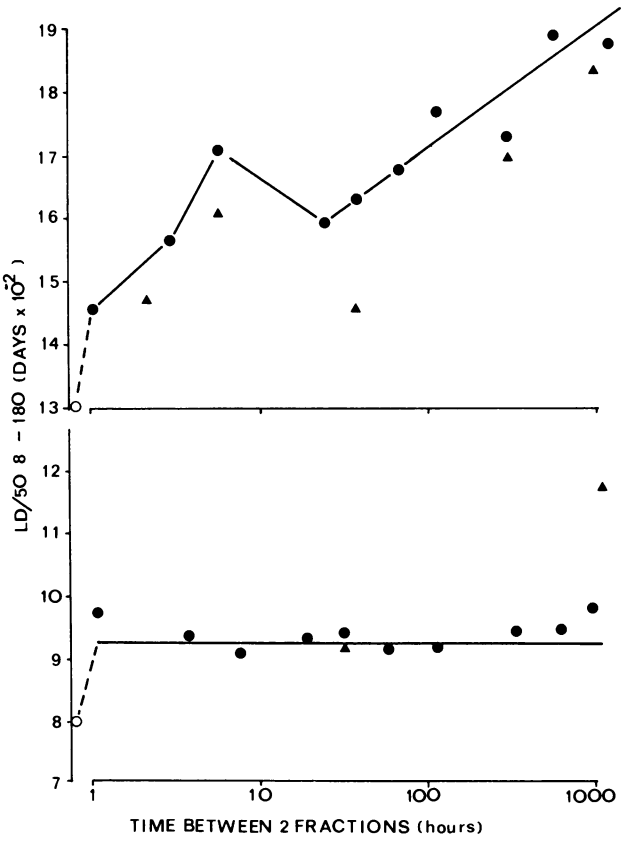

FIGURE 4. (Upper panel) the increase in LD_{50} for lung damage as a function of the time interval between two fractions of X-rays: (○) single dose; (●, ▲) data from two experiments; (lower panel) the LD_{50} increase for two fractions of neutrons: (○) single dose; (●, ▲) data from two experiments. From S. B. Field (*103*), courtesy of the author and Pergamon Press.

is an increase in the $LD_{50/180}$ for lung death with increasing time intervals between fractions (*104*). The most dramatic changes occur over the first few hours, especially for X-ray doses. This has been tentatively attributed to the repair of sublethal damage in surviving cells. For time intervals greater than 6 hr between X-ray doses, the $LD_{50/180}$ is increased by some 0.08 Gy per day up to 28 days and this is attributed to a "slow repair" process (Fig. 4, upper panel). For fractionated neutron doses (Fig. 4, lower panel) there is no evidence of the "slow repair" component, and there is a much reduced rapid recovery phase.

The RBE for a single dose of neutrons, for the $LD_{50/180}$ endpoint, is 1.5, i.e., 7.8 Gy neutrons produces the same effect as 11.6 Gy of X-rays.* The additional dose required in two fractions to produce the same level of damage as a single dose—the D_2-D_1 at 24 hr—was 4 Gy for X-rays and 2 Gy for neutrons.

Inhalation of large doses of alpha- and beta-emitting radionuclides causes death in experimental animals from both pneumonitis and fibrosis (*105,106*). Studies in beagle dogs have involved such beta-emitters as ^{90}Y, ^{91}Y, ^{144}Ce, and ^{90}Sr attached to fused aluminosilicate particles (FAP) to look into the effects of dose rate on the time and mode of pulmonary death (*107*). The more protracted the exposure the longer was the survival time of the exposed animals. For example, brief exposure to ^{90}Y caused death between 30 and 200 days with 50% mortality occurring after a cumulative dose of ~100 Gy. Whereas more protracted exposure to ^{90}Sr deaths occurred between 180 and 450 days after cumulative doses of ~560 Gy. Similar data have been obtained for acute death from pneumonitis and fibrosis in dogs after inhalation of the long-lived alpha-emitters $^{238}PuO_2$ and $^{239}PuO_2$. The doses for 50% mortality were 80 and 210 Gy, respectively (*106*).

Using the $LD_{50/180}$ system, compelling evidence has been reported for the primary role of type II cells and against the role for endothelial damage as the cause of death from radiation pneumonitis (*72*). The studies involved irradiation of mice either 2 or 6 days after treatment with butylated hydroxytoluene (BHT). At 2 days after BHT the proliferation of type II cells was at its peak and at 6 days endothelial cell proliferation was predominant. The $LD_{50/180}$ for X-rays alone was 9.6 Gy, which was reduced to 2.7 Gy when 2 days elapsed after BHT treatment and was increased to 14.5 Gy for mice irradiated 6 days after BHT. Similarly for fission neutrons alone the $LD_{50/180}$ was 4.8 Gy, and 2 and 6 days after BHT was 1.0 and 5.8 Gy respectively. Furthermore, there was a dramatic reduction in the time of death of mice irradiated 2 days after BHT: mice receiving only X-rays died between 140 and 180 days, whereas mice X-irradiated 2 days after BHT died of the same lung lesions at 18–40 days post-treatment. A similar shift in time of death patterns was observed for neutrons. The apparent increase in the $LD_{50/180}$ doses in the mice irradiated 6 days after BHT can also be explained by type II hyperplasia, i.e., there was an increase in the number of target cells, which accompanied the BHT treatment (*72*).

Finally, there is evidence to suggest a dissociation of early (pneumonitis) and late (fibrosis) damage in the lung.

These findings raise the question to what extent pneumonitis and fibrosis are distinct types of injury possibly with different target cells. The critical role of type II cells in acute pneumonitis has been discussed. It may be that pneumonitis and chronic fibrosis should not be viewed, as is often the case, as a continuum. Early damage may not therefore be predictive of late damage especially if the mechanism of the former involves type II cells and the latter involves endothelial damage and the late impairment of vascular function during late-fibrosis.

Clinical Pulmonary Lethality

The relevance of the animal lethality studies just described lies in the need for a clearer understanding of

*RBE is the biological efficiency of the radiation under investigation compared with that of therapy X-rays, under the same experimental conditions.

pulmonary toxicity after both simple radiotherapy and combined modality therapy. The severity of the injury to the lung, which impairs ventilatory and diffusion capacity, is related to the volume of tissue irradiated and to the tissue irradiated and to the fractionation and to the total dose.

The time course of the histological and functional changes in man and experimental animals is similar, although the single X-ray dose required for severe effects in the mouse (~10–12 Gy) is about 40% greater than that in man (~8 Gy). In patients, acute pneumonitis usually occurs 3–4 months after irradiation with the rapid onset of such symptoms as a nonproductive hacking cough, spiking fever, shortness of breath and even respiratory distress. Such symptoms occur in some 10% of patients receiving standard fractionated therapy regimes that involve one-third to one-half of one lung receiving a total dose of ~40 Gy over 4–6 weeks. A table of the predicted risk of pneumonitis as a function of the total dose and the number of fractions has been published (*40*). The increasing practice of upper half-body irradiation is an excellent palliative in cases of disseminated malignant disease, but carries a much higher risk of pulmonary complications because the whole lung is involved. Mortality occurs in a few percent of patients (*108,109*).

It has been accepted for some time that the clinical threshold for single X-ray doses, corrected for tissue inhomogeneity, is 6–7 Gy (*108,111*). Such a threshold is in agreement with predictions from mouse models by Wara and co-workers (*112*) that significant clinical complications might be expected for single doses of 7 Gy. These predictions have recently been strengthened (*109,110,113*) and the first attempt has been made to relate the incidence of pneumonitis to the absolute dose to the lung in patients receiving large-field radiotherapy (*114*). Since the survival time in advanced cancer patients is similar to the time of onset of pneumonitis, actuarial corrections have to be made for the early death of patients. The data given in Figure 5 show the dose to produce a given actuarial incidence of pneumonitis. The high incidence (53%) for the dose interval centred on 8 Gy looks serious when taken on its own but, as the report notes, when taken with the rest of the data this incidence is statistically not very significant. The steeply rising sigmoidal incidence curve shows that for single fractions the onset of pneumonitis occurs at 7.5 Gy with 5%, 50% and 95% complications occurring at 8.2, 9.3, and 10.6 Gy, respectively. These data are in agreement with the earlier reported uncorrected dose incidence data for patients receiving upper half-body irradiation (*110*). There are no data for man for the acute pulmonary effects or lethality from radionuclide exposure.

Current Concepts of the Pathogenesis of Radiation Pneumonitis

Gross (*39*) outlines a sequence of steps to explain the mechanisms underlying radiation pneumonitis. In essence, it is suggested that the primary response is an increase in the microvascular permeability, which causes an excessive leakage of plasma proteins onto the alveolar surfaces. This exudate overwhelms the lymphatic and other drainage mechanisms and elicits a secondary response from type II cells. There is type II cell hyperplasia and an increase in their lamellar bodies, accompanied by an increase in surfactant production as the lungs attempt to maintain normal alveolar surface tension. In the acute phase of pneumonitis, this homeostatic response of the type II cells is ultimately inadequate and the loss of surface-tension-lowering-forces ("surfactant deficiency") results in further edema and atelectasis. These in turn account for the fall in compliance, abnormal gas-exchange values and even respiratory failure that presage death.

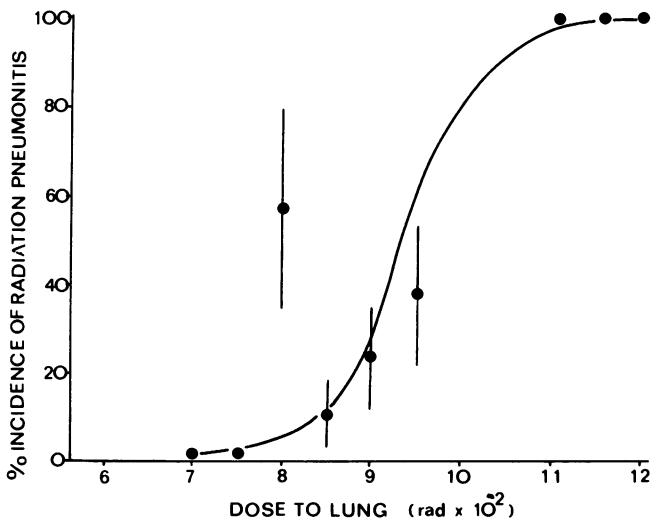

FIGURE 5. Best-fit sigmoidal curve of the incidence of radiation pneumonitis in radiotherapy patients. From J. van Dyk et al. (*113*), courtesy of the authors and Pergamon Press.

A number of points can be made about this postulated mechanism. While early vascular changes have indeed been reported (*8,18,49,59,60*), Gross (*8*) stresses that capillary permeability and protein leakage in his experiments appears relatively late at 12–16 weeks post-irradiation. This does not seem consonant with the idea that the vascular response is the primary response.

Again, as Gross notes (*39*), there is histological and biochemical evidence for increased surfactant production well before the onset of pneumonitis. This has been confirmed by recent biochemical studies in mice that showed a 3- to 4-fold increase in surfactant phospholipids in alveolar fluids within 4–6 weeks of thoracic X-ray doses of 10-15 Gy (*56*). This early wave of surfactant release paralleled a similarly timed wave of excess serum protein levels in the air-spaces. However, during the critical pneumonitis phases at 3 to 6 months, there was no evidence of enhanced surfactant levels to cope with the 10- to 20-fold increases in serum protein levels, i.e., during pneumonitis there seems to be no homeostatic response by type II cells as far as surfactant syn-

thesis and release is concerned (*56*). It has been suggested that at this time, radiation-induced type I cell death might trigger type II cells into division rather than into surfactant production.

There are data on the efficiency of corticosteroids in reducing the morbidity and mortality of radiation pneumonitis (*3,114–116*). It appears that such drugs have little effect on capillary leakage but act by causing a large increase in surfactant production, sufficient to offset the life-threatening mechanical and physiological effects of excess protein in the air spaces (*116*). However, the question as to why type II cells require an exogenous stimulus before they synthesize and release adequate amounts of surfactant remains unanswered.

The hypothesis outlined above seems to fit some though not all of the experimental and clinical literature. As a result, the pathogenesis of radiation pneumonitis remains a puzzle of cause and effect.

Late Effects of Radiation in the Lung

Pulmonary fibrosis and carcinogenesis are the major, late consequences of radiation, both clinically and experimentally. While fibrosis may not be completely separate from early pneumonitis, carcinogenicity can be regarded as an entirely separate phenomenon, if only for the considerably lower doses required for its induction.

Radiation-Induced Pulmonary Fibrosis

Introduction

The acute radiation injury described above is due to cell death and the depletion of cell populations. If the attempted regeneration of the parenchyma is inadequate, a final phase of pulmonary fibrosis may occur at periods in excess of 6 months after irradiation. Fibrosis has been described clinically and experimentally in many studies after both external and internal radiation. Acute X-ray doses to produce significant fibrosis in animals and man are generally higher than the $LD_{50/180}$. For example, in man, localized doses greater than 20–30 Gy are needed for significant fibrillar connective tissue deposition although mild focal fibrosis has been reported after $\geq$ 5 Gy (*3*).

The term "fibrotic" is a morphological one used to describe an increase at the light microscope level of connective tissue fibers in response to some insult (Fig. 6). The pathologist is aided by the characteristic staining properties of the collagenous components of the fibrous tissue. Coarse-fibered collagen has affinities for basic aniline dyes in acid solutions (*117*), while finer meshworks can be identified with the so-called reticulin stains. It is now becoming clear, however, that the changes in pulmonary fibrosis are much more complex than a simple increment in collagen fibers (*118*), even if this is described biochemically.

Radiation-induced pulmonary fibrosis should not be considered in isolation: it is just one example of a heterogeneous group of chronic disorders in which there is fibrosis of the alveolar structures. Recent reviews have summarized the available information on these conditions (*118,119*). From the results of these studies several changes can be described, in addition to an increased prevalence of collagen fibers.

The collagen accumulated during fibrosis may alter the normal ratio of Type I (coarse fibred) to Type III (meshwork) collagen. Early studies involving collagen-typing were beset by problems of extraction of the macromolecules from lung tissue. With relatively low recoveries, the collagen types within the extract may not have been representative of the tissue as a whole. Recent work by Seyer and co-workers (*120*), using cyanogen bromide cleavage of whole lung samples, has demonstrated an increase in the proportion of Type I collagen in the lungs of patients with idiopathic pulmonary fibrosis (IPF). However, others have reported a slight, early increase in Type III collagen in IPF (designated by them as cryptogenic fibrosing alveolitis—CFA) (*121*), and samples of lung from patients with progressive systemic sclerosis with pulmonary involvement, has unchanged Type I:Type III collagen ratios (*122*).

The amounts of elastin and the connective tissue matrix material—glycosaminoglycans—may also change during fibrosis. Changes in the proportion of the latter seem to parallel that of collagen, with the increase equally distributed among all glycosaminoglycan subtypes (*123*).

Cellular disturbances play an important role in the alterations or even destruction of the alveolar architecture. These include cell death on both the endothelial and epithelial sides of the basement membrane, with interruptions, layering and thickening of the membrane itself. In addition, the process always appears to involve effector cells of the immune system, often as part of inflammatory and infiltrative phases (*124*). As a result, cellular infiltrations of the alveolar spaces, e.g., following bleomycin administration (*125*) or in the adult respiratory distress syndrome (ARDS), have been described as "fibrosis." However, the connective tissue changes may be relatively small in comparison with the cellular ones and therefore these conditions may be more akin to the pneumonitic phase of radiation damage than the fibrotic one (*38*).

Radiation pulmonary fibrosis includes all the facets described above, although, as will be shown, as a model of fibrosis it has not been studied in as great detail as, for example, IPF or silicosis. As described above, it contains one distinctive feature: within the pneumonitic phase there is a distinct latent period. It is this phase that intrigues and baffles researchers, yet may in the future hold the key to understanding the fibrotic process in general.

The Clinical Significance of Fibrosis. Several reviews have been produced on the pulmonary side effects of radiation therapy, for example: Gross (*38*), van den Brenk (*14*), Phillips and Wyatt (*40*). By stressing the severe complications that pneumonitis may produce, authors have placed late radiation fibrosis in perspective.

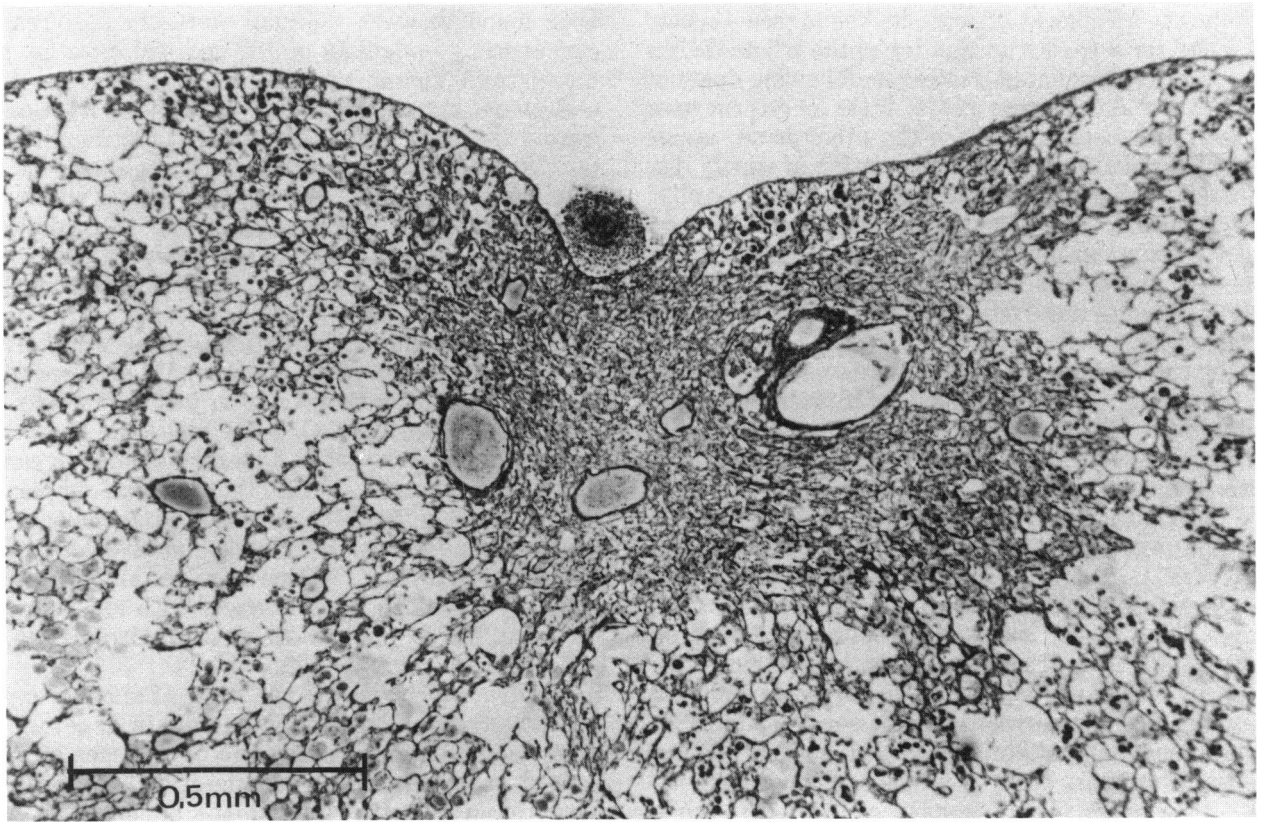

FIGURE 6. Radiation-induced fibrotic nodule in mouse lung, 14 months after inhalation of $^{239}PuO_2$ (silver-reticulin stain).

Although fibrosis is a very common consequence of therapy, the induced clinical symptoms are usually minimal—merely a mild deterioration in pulmonary function (*38*).

Biochemical and histological evidence of fibrotic changes may be present as early as two months after exposure. The progress of the lesion, as followed by radiology, is insidious over 1–2 years, by which time a stable state is reached (*38,40*). During this period any pneumonitic changes have been followed by a reparative phase, with organization of dead cells and inflammatory exudates. Such changes are often associated with radiological or tomographic evidence of resolution within the lung (*14,126*), although the affected areas become more sharply defined, as permanent fibrosis replaces the cellular infiltrates. As such, this may be regarded as the delayed manisfestation of cell death, with secondary waves of repair, often associated with devascularization of the fibrotic area (*14*). Phillips and Wyatt (*40*) reported fibrotic changes in most patients who had received a dose that "just suffices to cause radiation pneumonitis" while Gross (*38*) described long-term changes in the majority of patients, whether or not pneumonitis had preceded them.

It is usually only secondary complications that are of clinical significance. For example, cystic or bronchiectatic changes sometimes occur in large zones of fibrosis—there may be pleural effusion, a spontaneous pneumothorax or even a bronchial obstruction as a result of the collapse of a tumor site (*40*). However, the main effect of the fibrosis is associated with a reduction in lung volume as the affected region contracts. This may cause a shift of the mediastinum towards the irradiated side, with deviation of the trachea (*40,126*). Deeley (*127*) reported evidence of lung shrinkage in all surviving patients, 15 months after external high-energy X-ray therapy for inoperable bronchogenic carcinoma. Investigation indicated that there was extensive fibrosis at the site of the primary lesion, surrounded by macrophage infiltration and exudation into the alveolar spaces. Marked movement of the trachea and heart was often found, helped by compensatory hypertrophy of unaffected regions of the lung. The fibrosed lung was prone to infection, which could exacerbate cardiac problems induced by pulmonary vascular deficiency. Indeed, right heart failure may assume a major complication in such extreme cases (*128*). Further progression to "cicatrisation" has been described by van den Brenk (*14*) with even calcification and ossification becoming evident in the devascularized regions. However, such changes are rarely reported in the literature and may represent the endpoint for highly focal irradiation of parts of the lung at high dose rates.

Indeed, high dose rate may be a critical factor in the production of late pulmonary effects (*129*). For example, during total body irradiations (TBI), used in recent

years for the treatment of acute leukemia [see Thomas et al. (*130*) for a review of this topic] the whole thorax is, necessarily, irradiated. However, following doses of 7.5 to 12 Gy at low dose rates, little long-term lung pathology has been found. On the other hand, upper half-body irradiations, for the alleviation of widely disseminated malignant disease, while using a similar range of doses to TBI (6.5–12.5 Gy) have been given at up to 4 Gy/min. In many cases, radiation pneumonitis has been a major complication of the treatment (*113*), which brings with it the possibility of fibrosis developing at a later stage.

As the gas-exchange interface is affected by fibrosis, with thickening of alveolar-capillary barriers and a reduction in the effective surface area, so gas transfer is impaired. However, the first and predominant effect of fibrosis in man is to produce an abnormally "stiff" lung (*119*). Both static and dynamic lung compliance are reduced and may be accompanied by a reduction in vital capacity (*127*). Because of the increased effort required, higher breathing rates are adopted with smaller tidal volumes. As a result, a smaller proportion of inspired air reaches the alveoli and thus the minute volume may also be increased. It should be noted however, that there may be little correlation between lung function measurements and either histological or biochemical evidence of fibrosis. Early compliance changes are associated with edema (*131*) or surface effects (*38*), while only at later times is a "tissue element" involved in the stiffness (*38*). In addition, paracicatrical emphysema may develop around incompliant, fibrotic areas giving virtually normal physiology, but with gross pathology (*131*).

Experimental Studies of Radiation Fibrosis. Since both pneumonitis and fibrosis are consequences of therapy regimes, many animal studies have concentrated on the effects of external irradiation. The health protection needs of the nuclear power industry have led to numerous animal studies involving the effects of internally deposited radionuclides. However, the initial alveolar depositions (IADs) required to produce pulmonary fibrosis probably exceed the levels that could be achieved in any maximum credible accident. Nevertheless, they may lead to an understanding of the mechansims of fibrosis.

EXTERNAL IRRADIATION. Using whole-body plethysmography, Travis and co-workers (*132*) demonstrated two phases of reaction following X-irradiation of the whole thorax of mice, with peaks in breathing rate at about 16 weeks and 36 weeks, respectively. The first phase would probably correspond to the acute, inflammatory phase of pneumonitis, which had been reported separately (*133*). At 36 weeks, in animals surviving the earlier pneumonitis, the lungs would have shown greater cellularity, with both pathological organization and increased amounts of collagen fibers (*132*).

The increasing evidence to suggest a dissociation between early pneumonitis and late fibrotic changes in the lung was noted above. Not only is the histological picture very different (*73,132*) but a clear separation has been found between the lung mortality dose/response curves using endpoints of 180 and 420 days for pneumonitis and fibrosis, respectively (*74*). Further, Travis and Down (*73*) showed that while dose fractionation spared the pneumonitic phase it did not diminish late, fibrotic changes. More recent work has shown that one strain of mice (C57/B1) develop late effects without any evidence of acute pneumonitis (*134,135*). In addition, it has been shown that the radioprotective agent WR2721 is considerably more effective against the later effects (*136*).

With whole-thorax irradiation of rats, Kurohara and Casarett (*13*) could show pneumonitic changes (designated "fibrosis" by them) within just 4–5 weeks after supralethal doses (24 Gy). After moderate doses, however (6–12 Gy), the lungs appeared similar to controls for at least 35 weeks. At the other extreme, upper-body exposure of dogs to 21 Gy of X-rays (*137*) produced many deaths from "pulmonary fibrosis and alveolar-capillary block" within a year. The survivors and animals exposed to 18 Gy produced some fibrosis, with associated emphysema, but due to the design of the irradiation, also suffered from thyroid dysfunction.

Biochemical changes in the lungs of animals exposed to whole-thorax irradiations do not, in general, show clear demarcations between the two phases of injury. Indeed, many of the changes described as fibrosis apparently have their origins in the pneumonitic phase. In mice, lung weight has been shown to increase within 18 weeks of exposure (*138*), which may be reflected in collagen content, as determined by hydroxyproline assay (*139,140*). Similar results have been obtained in rats by Dubrawsky and co-workers (*141*). When expressed as concentrations in the lung, hydroxyproline was linearly related to dose over the range 7.5 to 15 Gy, but as lung weight did not always increase, these changes may have been more complex. In general, it is more usual to have clear threshold effects for both pneumonitis and fibrosis, at values which are close to the $LD_{50/180}$ dose (*142*).

This effect is demonstrated in Figure 7 from studies carried out in our own laboratories. Groups of 20 female SAS/4 mice from a randomly bred colony were exposed thoracically to X-rays, to give whole thorax doses of 5–15 Gy. The total collagen content of the lungs was only elevated significantly after 15 Gy, a dose slightly above the $LD_{50/180}$ one of 12 Gy. It is clear that the response to 5 Gy was negligible, while following 10 Gy the initial increase in collagen had not become progressive within the 9-month experimental period.

Studies in animals involving irradiation of the whole lung are therefore far from straightforward and are by no means complete. At the moment, two separate phases can only be described in mice, and even then, the biochemical data are sparse.

Whole-lung irradiations simulate hemibody and TBI therapy. Any ensuing fibrosis, which would involve the whole lung, may be analogous to chronic interstitial fibrosis produced by a number of other agents, for example asbestos, silica, or paraquat (*119*). Partial irra-

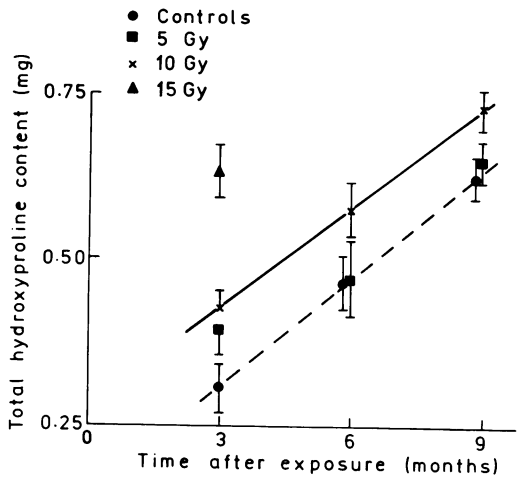

FIGURE 7. Total amount of pulmonary collagen, as shown by determination of hydroxyproline, at various times after whole-thorax X-irradiation of 6-week-old mice (means ± S.E.M.).

diation of the lung is clinically more common as a consequence of localized treatment of carcinoma in the thoracic region. However, both clinical and experimental evidence indicates that the reaction of the lung is rather different.

This is well illustrated by the work of Law and coworkers (*143*). Mice were given hemithoracic irradiations of 10–40 Gy X-rays. Between 24 and 36 weeks, after exposures in excess of 20 Gy, the concentration of hydroxyproline in the irradiated lung increased. This has been confirmed by several other workers in both rats and mice, at doses above 10 Gy to one lung (*5,35,37,144,145*). However, the data of Law et al. also imply that the total hydroxyproline of the irradiated lung actually decreased after exposure above 20 Gy. After a 10 Gy hemithoracic exposure, the total amount of collagen in the irradiated lung, as determined by hydroxyproline, increased gradually with time.

An extreme example of this effect was demonstrated by Collins et al. (*146*) following 30–40 Gy ^{60}Co gamma-irradiation of the upper parts of baboon lungs. A "fibrotic" condition was described at 6 months after exposure, with increased collagen concentrations in the affected regions. However, total collagen had, like other lung components, actually decreased in the irradiated areas. As collagen was not lost as fast as other components, its concentration appeared to increase. As the irradiated areas shrink with time, this extreme case may be more of a "radiation stump" than a true fibrosis. Following highly localized irradiation of rabbit lungs at high dose rates and doses, calcium deposits and even new bone formation have been reported (*147*). In these circumstances, the shielded regions of the lung may not only compensate for the functional loss of the irradiated areas but, as has already been described (*40,127*), may physically replace shrunken areas by a compensatory hypertrophy. As a result, the shielded region is no longer a valid contralateral control.

The combined effects of decreased function, slight atrophy, and compensatory hypertrophy of the unaffected regions, therefore produce an effect that is not completely analogous to other chronic interstitial fibroses. However, the long-term pathological description will frequently be of "fibrosis" and be biochemically indistinguishable from the whole-lung form. Local fibrosis, where the insult has been administered to only one part of the lung, may produce a rather different final expression of the lesion. Rather than the discrete foci that distinguish the fibrosis resulting from whole thorax irradiations, local fibrosis includes a tendency to atrophy as the unaffected region is able to compensate for the initial damage. As commented elsewhere, localized effects in which a "radiation stump" ensues may be beyond the upper limit of pulmonary fibrosis (*119*).

Studies on localized radiation fibrosis, while showing some dissimilarities do, however, indicate many aspects common to whole-lung exposures. Indeed, the dose-response characteristics of changes in breathing rate following hemithoracic or whole-lung irradiation of mice have been shown to be identical (*131*). Two phases of damage have been described histologically (*148,149*), and an important, although as yet not fully defined, role for mast cells in alveolar walls has been described, prior to the second, fibrotic phase (*148,150*). The changes in the alveolar region were mostly epithelial, with less pronounced effects in the pulmonary vasculature (*148*).

During both the pneumonitic and fibrotic phases of localized lung damage, the metabolism of connective tissue components is grossly disturbed. Using the extractability of collagen from lung in salt or acid solutions as a measure of recently synthesized material, Bublitz (*151*) reported evidence of increased collagen synthesis during pneumonitis in the irradiated part of the lung in rats. This was accompanied by increased concentrations of glycosaminoglycans within the so-called latent period, i.e., by 8 days after exposure. A wave of collagen synthesis has also been described in the lungs of hamster after fractionated, hemithoracic ^{60}Co gamma irradiation (*149*). An increase in soluble collagen was evident at 21–22 weeks after exposure, although enhanced collagen synthesis, as indicated by the incorporation of tritiated proline, peaked some 6 weeks previously. An 8-week latent period was evident in these experiments, and, after an inflammatory pneumonitic phase at 14 weeks, fibrosis—as defined both histologically and biochemically—was evident by 36 weeks.

Disturbances of connective tissue metabolism have also been seen by transitory decreases in pulmonary hydroxyproline concentration soon after hemithoracic irradiations (*5,144*), prior to the later, incremental, phase of fibrosis.

INTERNAL IRRADIATION. The radiological dose to lung tissue resulting from the inhalation of radionuclides is a function of the physical half-life of the isotope and its residence time in the lung, usually defined by a half-time of clearance. As a result, the dose is usually protracted over days or even many years. This complicates the analysis of the sequence of events, but it does allow

whole-lung irradiation, without necessarily producing major problems of early mortality.

Several studies have used virtually insoluble, fused aluminosilicate particles (FAP) to carry low LET, beta-emitting radionuclides to the parenchymal regions of the lung. In this way, relatively uniform, highly protracted irradiation of the lung can be achieved. Using short-lived ^{90}Y, research at the Inhalation Toxicology Research Institute (ITRI) in Albuquerque demonstrated a typical pneumonitic phase in beagle dogs (*150*) and lung function changes typical of pneumonitis and fibrosis (*153,154*). These workers emphasized that fibrinolysis was an integral part of the inflammatory phase, linking this work to the X-ray studies of Gerber and co-workers, who have also demonstrated the significance of biogenic amines in the maintenance of inflammation (*144*).

The most significant aspects of the connective tissue studies of the ITRI group are their extensive studies of collagen metabolism. They demonstrated an early increase in ultrafilterable hydroxyproline in the lungs of exposed animals (*155*). This occurred during an early period of low static compliance in the lung, when pneumonitis was prevalent. It has been interpreted as an increase in collagen degradation, preceding the synthetic phase that produced more soluble collagen (*149,155*) and increased incorporation of labeled proline as hydroxyproline (*156*). Later studies showed that, at the high accumulated doses required to produce fibrosis, the initial dose rate was critical (*107,154,157*), as summarized recently by Pickrell (*119*).

The effects of alpha-emitting radionuclides have been studied extensively in laboratory animals. As potential risks in the nuclear power industry, insoluble alpha-emitting particles have come under close scrutiny as the dose delivered by them to the surrounding tissue is extremely inhomogeneous. This has implications for dose calculations and commitments, especially related to neoplastic changes.

The early studies of Battelle-Northwest Laboratories and others have been summarized by Bair (*158*), allowing broad guidelines to be established. At initial concentrations of alpha-emitting plutonium in the lung in excess of 20 MBq/kg (0.5 μCi/g), death due to pneumonitis occurs relatively quickly (*57,106*). Below about 2 MBq/kg, the animals' survival time is only marginally affected, and long-term neoplasia may be induced. Between these two extremes, plutonium-induced fibrosis will be induced, its time of onset being dependent on the initial concentration of the actinide. These can, however, be only taken as the crudest guides, as isotope and chemical form affect clearance rates. As a result, both dose-rate and total accumulated dose to the lung would be affected.

The connective tissue studies of the ITRI group in hamsters (*154,159*) are at the lowest end of this range (2.5 to 5 MBq/kg). Their results are summarized in Figure 8, for an IAD of 4 kBq of 238-plutonium dioxide ($^{238}PuO_2$). The dose from the insoluble $^{238}PuO_2$ particles would have been delivered relatively slowly—about 0.2

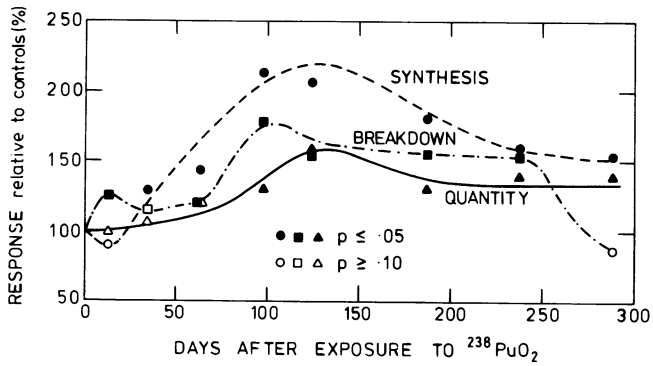

FIGURE 8. Pulmonary collagen metabolism in hamsters as a function of days after exposure to 3.7 kBq $^{238}PuO_2$. Fraction proline incorporation indicates (○,●) relative synthetic rate; (□,■) ultrafilterable hydroxyproline, collagen breakdown; and (△,▲) quantity, collagen accumulation or scarring. From Pickrell et al., (*159*), courtesy the authors and the Lovelace Foundation.

Gy per day—decreasing over the course of the experiment. The sequence of connective tissue changes was protracted producing the most clearly demarcated biochemical phases of all the radionuclides studied (*154*). They began with increased collagen breakdown, associated with a neutrophil infiltration, preceding both synthesis and the resulting increment in total quantity. The accumulation of collagen was greatest at about 130 days, when parenchymal scarring was evident. Although collagen synthesis returned to more normal levels by 290 days, these scars, with their concomitant collagen, remained in the lung producing pulmonary failure. The distribution of the scars has been shown to be dependent on foci of $^{238}PuO_2$ particles, around which the collagen is accumulated (*160*).

Some studies with 239-plutonium, which has a lower specific activity (longer half-life) than the 238-isotope, have used IADs high enough to promote an early pneumonitic response, with either insoluble $^{239}PuO_2$ in hamsters (*161*) or a variety of more soluble forms, in rats or rabbits (*162–168*). Such studies have described some of the cellular changes during both pneumonitis and fibrosis (*161,162*) and have shown that the metabolism of collagen (*160,164–166*), glycosaminoglycans (*163,165*) and lipids (*162,166*) are all shifted towards enhanced synthesis. Within the glycosaminoglycans, radiation fibrosis alone (*123*) appears to selectively involve certain sub-types including chondroitin and heparin sulfates after both internal (*163*) and external (*169*) irradiation.

A rather different sequence of events has been described by Metivier and co-workers (*170–172*) after inhalation exposure of rats to high IADs of $^{239}PuO_2$ (5–6 MBq/kg). They reported a biphasic response, with an initial peak of fibrosis, as seen both by histology and analytical biochemistry, at about 230 days, a partial return to the biochemical values of controls at 400 days and signs of a secondary wave of fibrosis at 500 days. The recovery phase at 400 days was not reflected in histological improvement and was not apparent in animals dying over the same period (*170*). It would, there-

fore, appear that a "survivors" effect may have been responsible for much of the apparent recovery (*173*), especially as later studies compared groups with dissimilar IADs at different times, so that they would have accumulated similar total doses (*171*). The system has also been used to demonstrate metabolic changes by "biochemical dissection" (differential extraction), which imply a major role for catabolism in the control of collagen concentration in the lung (*172*). These studies were also characterized by a biphasic response.

However, in subsequent studies with mice in our own laboratories, no real indication of a recovery phase could be demonstrated (*174*). As is shown in Figure 9 the lungs of mice were analyzed at various intervals after exposure to sized $^{239}PuO_2$ particles, to give IADs of 6, 42, and 925 Bq (equivalent to 0.04, 0.3, and 6.2 MBq/kg, respectively). Figure 9 shows the collagen response, in terms of hydroxyproline content, relative to the lungs of simultaneous control animals. Only the highest IAD produced significant changes, with a 40% increase in lung collagen at 3 months, with only slight changes over the next 9 months. Thereafter, a second phase was evident, which was associated with the accumulation of fibrotic nodules and associated cell infiltrations. The response up to 9 months was reminiscent of the process described for hamsters after $^{238}PuO_2$ exposure (*154*), with an additional secondary phase missing from those shorter studies, but demonstrable in rats at 500 days (*170*).

So, a pattern of radiation fibrosis is emerging, consisting of an initial increase in collagen degradation, followed by a much larger increase in collagen accumulation. Whether there is a causal link between the two and the actual site of the degradation, still has to be determined. In the same way, although collagen builds up during fibrosis, the mechanism—whether by enhanced synthesis or reduced degradation—has still to be elucidated. Although enhanced incorporation of

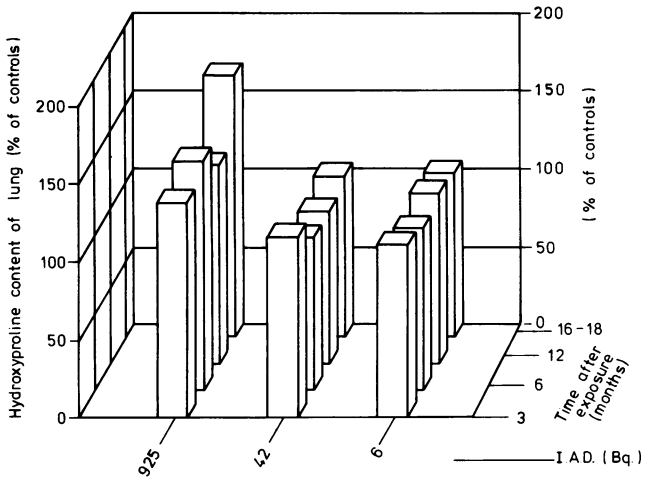

FIGURE 9. Total amount of pulmonary collagen, as shown by determination of hydroxyproline, at various times after inhalation exposure to $^{239}PuO_2$ of 6-week old male and female SAS/4 mice to give three initial alveolar depositions (*174*).

radiolabeled proline into hydroxyproline has been reported frequently, the techniques used would not have compensated for any changes in the metabolic pool size in the fibrotic lung, a feature common to more recent methods (*175*). The type of collagen accumulated in radiation fibrosis is also unknown. Other fibrosis studies, using elegant techniques, have found little change in collagen type during chemical-induced fibrosis (*176,177*). It is possible that it is the inappropriateness of the site of the new collagen that is wrong.

In summary, while attention to breathing rates and disputes over collagen metabolism have concerned radiobiologists in this field for several years, the pathogenesis of radiation fibrosis remains virtually unknown. Classical theories of fibrosis that involve macrophage recruitment and damage (*178*) seem inappropriate when, as has been shown above, macrophage numbers may be severely depleted (*11,179*), especially during the chronic damage induced by inhaled $^{239}PuO_2$ (*180*). Indeed, the chronic nature of this damage may be the key element, in the same way that chronic inflammation is a major feature of mineral dust-induced fibrosis (*124*).

The maintenance of inflammation is an important side-effect of fibrinolysis during pneumonitis (*144,152*) and some form of prolonged inflammation has been recorded as part of almost every acute response to radiation by the lung. Even following the low dose-rates of $^{239}PuO_2$ to the lung, when little edema or leukocyte infiltration can be seen (*170,174*), the macrophage depletion itself may be a manifestation of chronic damage by phagocytosed alpha-emitting particles.

Speculation over the sequence of events in radiation pneumonitis and fibrosis may seem premature, but if it is made in the context of fibrosis in general, it may be valuable. In attempting such a synthesis of ideas, Pickerell (*181*) has highlighted the integrity of the basement lamina as crucial in delineating reparable from disruptive and ultimately, scar-producing lesions. The exposure of basement lamina can occur in many ways, including, of course, by both endothelial and epithelial damage. With protracted radiation, the chronic stimulus producing denudation and lysis is obvious. In contrast, in fibrosis after external exposure at high dose rate, a chronic component appears to be absent: fibrosis develops only after a characteristic latent period. However, such delayed "avalanche" expressions of radiation damage are consistent with recent theories of cell kinetics in which, for example, the lung would appear to be closest to the F (flexible) type, in which all cells have the potential for proliferation (*182*). As a consequence, the more severe damage of large doses is seen before that produced by smaller doses. There is, however, a limit to the minimum time before manifestation of lung damage (*142*), when perhaps a critical proportion of essential cells (for example, Type II pneumonocytes) have ceased to function (*183*). Such hypotheses have been constructed primarily to explain the pathogenesis of pneumonitis, but they are also compatible with late fibrosis. For example, in the fibrosis induced in dogs by inhalation of beta-emitting particles, the animals died

with exactly the same fibrotic lesion, but at different times after exposure (*157*). A "preclinical" stage of development was advocated, with variable length, followed by the development of the lesion over a fixed time sequence. On an individual basis, this is equivalent to the latency exhibited by, for example, X-irradiated populations of mice but without the uniform time to appearance of the lesions that characterize high "instantaneous" doses.

Radiation-Induced Pulmonary Cancer

Human Data. Of the neoplasms induced by radiation, lung cancer was the earliest to be recognized, and it is one of the most important because of the very high mortality associated with the disease. In man, several populations have been studied intensively, including the Japanese survivors of the U.S. atomic bombs, patients irradiated for either ankylosing spondylitis or tuberculosis and various groups of uranium and other hard rock miners (*184,185*). The miners received their irradiation primarily from the inhalation of airborne alpha-radiation, while the other groups have been exposed to external radiation.

Radioactivity in mines is due to the diffusion of radon and thorium gases, derived from uranium-238 and thorium-232 in igneous rocks. Obviously the greatest levels and therefore hazard are found in uranium mines, but significant concentrations do occur in other types of mine. Radon-222 diffuses from the rocks and soils into the air and rapidly decays (half-life 3.8 days) to give radon daughters, two of which are isotopes of polonium. When these airborne decay products are first formed they are single ionized atoms, but they quickly attach to dust and water vapor to produce respirable aerosols. These are of such a size (~0.4 μm AMAD) that they can penetrate to the bronchi and beyond to be retained in the lungs.* High radon concentration was suggested many years ago as the cause of excess lung carcinoma in Schneeberg metal miners and Joachimstal uranium miners (*186,187*). More recently, a correlation between exposure and increased frequency of small-cell anaplastic cancers, together with excess numbers of epidermoid cancers and adenocarcinomas, has been reported for U.S. miners (*188*). There was no excess in these miners of large cell undifferentiated cancers and bronchoalveolar tumors. Horecek et al. (*189*) analyzed the histological types of bronchial cancers in Czechoslovakian uranium miners and confirmed the data on excess of epidermoid and small-cell undifferentiated cancers (*188*). Excess small-cell tumors have also been reported for South African uranium miners (*190*). There are minor epidemiological surveys from Canadian uranium and fluorspar miners (*191,192*), Swedish metal miners (*193*), and United Kingdom hematite miners, all of which support the relationship between excess mortality from lung cancer and internal radiation exposure (*194*).

It is difficult to separate the relative contribution of cigarette smoking and radon exposure in miners, because there are generally too few nonsmoking miners to make a control group. The UNSCEAR report of 1977 (*184*) stated that the data do not allow one to say categorically whether smoking has a cocarcinogenic, multiplicative effect or an additive effect. The 1980 BEIR III report (*185*) said that a multiplicative effect of smoking is highly unlikely. Nevertheless, there is some evidence for a cocarcinogenic effect between smoking and radiation with lung cancer rates up to ten times greater in smoking miners than nonsmoking miners (*195*). Further, it has been shown that induction-latent period for cancer in nonsmoking miners is longer than in miners who smoke 20 or more cigarettes per day (*196*).

Much effort has gone into evaluating the environment of uranium miners. Miners are exposed to external beta- and gamma-irradiation, to airborne radon daughters and to rock dust. The inadequate, infrequent or absent air sampling of some mines, especially prior to 1950, makes estimates of the cumulative doses only very approximate. However, compared with today, the dose rate under the early mining conditions was on average at least 10 working levels, i.e., 10 times the limiting dose rate in current practice (*194*).* Today, most countries accept the WL limit and try to ensure that no worker is exposed to more than 2 WLM in a consecutive 3 month period and no more than 4 WLM in any consecutive 12 month period. Modern mining conditions involve frequent air sampling, personal protective equipment and high ventilation rates. In addition, care can be taken in the separation of the radon ventilation system and radon-containing water, from main airduct and water supplies. All of these measures efficiently reduce the radon hazard.

The relationship between the WLM and the dose to the lung is complex, but it is generally agreed that 1 WLM delivers ~1 cGy of alpha radiation to the bronchial epithelium, the main target tissue (*184*). Using WL limits, several studies have shown that the relationship between the alpha dose and the excess mortality from lung cancer is consistent with a linear regression through zero (*184,185*). If it is assumed that 1 cGy of alpha radiation is equivalent to 5–10 cGy of low LET X- or gamma rays, then the risk of fatal lung cancer from exposure to low LET radiation would be between 20–150 cases per 10^6 persons exposed to 1 cGy (*184*). This estimate is in relatively good agreement with the values of 20 fatal cancers per 10^6 per cGy from Hiroshima and between 10 and 25 fatal cancer per 10^6 per cGy from Nagasaki (*184*). These risk estimates assume a linear dose-response relationship at least for high LET alpha radiation. The 1980 BEIR report (*185*) said that

*AMAD is the activity median aerodynamic diameter, that is, the median diameter, as a function of radioactivity, of a unit density sphere that would have the same terminal velocity due to gravity as the particles under consideration.

*A working level is defined as any combination of short-lived radon daughter products in one litre of air that will result in the emission of 1.3×10^5 MeV of potential alpha energy. Working level month (WLM) is the exposure to one working level for 170 hr.

not only is the newer information on lung cancer rates consistent with this assumption, but also the lowest dose at which lung cancer is increased has been lowered. However, the risk estimates for lung cancer given in that report are somewhat confusing and are not in agreement with those in UNSCEAR 1977 (*184*). They have been severely criticized as gross overestimates (*197*). It is suggested that the BEIR estimates are probably too high by a factor of 2–5 for miners. Because of different smoking habits, the risk to the public may be another factor of two lower.

The human evidence for the induction of lung tumors following external radiation comes from a number of sources. They include not only the victims of the atomic bombs (*184*), but also the mortality statistics of British radiologists (*198*) and the mortality of patients treated for ankylosing spondilitis (*199*).

In the next two sections we shall concentrate on the experimental studies that are most relevant to the human lung cancer data just described. The discussion is divided into experiments involving external irradiation and those involving irradiation from internal emitters.

Experimental Studies with External Irradiation. Most human lung cancer arises in the upper part of the bronchial tree, while most experimental animal tumors arise in the bronchioalveolar region. This fact is often used to question the relevance of animal models. On the positive side, there are numerous experimental studies that have helped to advance our understanding of the human lung cancer data. The following lists some recent advances and is taken from the 1980 BEIR Committee Report (*185*).

Respiratory tract tumors develop in animals exposed to radiation at sites where the local irradiation exposure is greatest. Bronchial and nasal sinus tumors have been produced in animals exposed to radon and its daughters. The effects of cigarette smoke on the development of bronchial cancers in radon experiments remain equivocal. The sensitivity of the respiratory tract of animals to cancer induction by radiation may be increased by irritant or other proliferative stimuli given after the radiation exposure. The bronchial tissue in the lungs is itself a separate compartment whose uptake and release of inhaled materials may play an important role in diseases such as bronchogenic carcinoma arising in the bronchial epithelium.

Most of the external irradiation experiments used mice and have been designed to study the details of dose-response relationship and to explore fundamental radiobiological mechanisms. A distinction needs to be made between experiments involving whole-body irradiation and those using localized thoracic exposure.

Many of the early experiments were broad-based and designed to study radiation lifeshortening and a wide range of somatic effects of uniform whole-body irradiation. Interpretation of the results is complicated by variations in the age at death, so that actuarial adjustments have to be made of intercurrent mortality. With such corrections, the results are still not in agreement: the majority of studies reporting a decreased incidence, some an increase and some no change in the incidence of lung tumors after radiation.

Thus two early studies both reported increased incidences of lung tumors in mice after whole-body irradiation (*200,201*), while an experiment to study the relative effects of X-rays and neutrons found lower incidences of tumors after both types of radiation (*202*). In this study, the control mice had a 24% incidence, whereas 8 Gy X-rays produced an 8% incidence and 2.9–5.8 Gy, 8 MeV neutrons produced a lung tumor incidence of 9% (*200*).

In LAF_1 mice exposed to whole-body gamma-rays from the explosion of an atomic device, the pulmonary adenomas appeared late in life. The incidence markedly declined with dose, when the figures were corrected for intercurrent mortality (*203*), although the induction-latent period was reduced by the radiation. Another study of LAF_1 mice also found a decreasing incidence of lung tumors after gamma doses up to 7 Gy (*204*). The slope was steeper in male than female mice, but overall, the males had five times the incidence of lung tumors than female mice. In another mouse strain, SAS/4, a decline in pulmonary tumors was reported over the dose range 0.5–4.75 Gy for mice irradiated with 14 MeV electrons, when correction was made for intercurrent mortality (*205*). In female RF mice, "no real increase" in lung tumor incidence was reported after 5–6 Gy X-rays, but there was some acceleration in the appearance of tumors (*206*). A decreasing incidence of tumors was produced after both 5 MeV and 14 MeV neutrons in female RFM/Un mice (*207,208*). In male RF mice, there were no differences between the effects of X-rays and neutrons both showing a decline with increasing dose (*208*). Protons also produced a decreasing lung tumor incidence with dose in RF mice (*209*). In a comparison of acute gamma irradiation with acute and protracted neutron irradiations, it was found that gamma-rays decreased the lung tumor incidence up to 1.5 Gy, but there was a slight increase at 3 Gy (*210*). Acute and protracted neutron exposures in these studies gave the same peaked response, with increased tumor incidences at doses as low as 0.2 Gy and a maximum incidence at 1 Gy (*210*).

It is obvious that no general conclusion can be drawn from this scatter of data. No single systematic study stands out and the inadequacy of these whole-body lifespan studies is related to (a) the death of animals prior to detection of lung tumors, (b) to difficulties of detecting tumors in animals after death, and (c) to the complications involved in the various age-adjustment procedures used to correct for mortality rates in different radiation groups.

Such considerations and criticisms led several groups to use localized thoracic irradiation. Once again, however, most of the experiments are too small and unsystematic to yield valuable data, although the overall results are more consistent than for whole-body exposures. Both rats and hamsters given fractionated thoracic doses of 35 and 40 Gy, respectively, developed sufficiently more squamous cell carcinomas compared with the zero incidence in control animals (*211,212*).

Single thoracic doses of 30 and 40 Gy in the same studies induced significant increases in lung tumors (*211,212*). A much quoted study reported in 1973 showed a very complete dose-response curve for lung adenomas induced in RFM mice 11 months after thoracic X-irradiation with doses between 7 and 30 Gy (*213*). The incidence peaked at 15 Gy, when 75% of mice had pulmonary nodules. Thereafter, the incidence then declined, reaching control levels at 27.5 Gy. The mean number of adenomas per mouse also showed a markedly peaked response, which closely paralleled the peaked incidence-response curve (*213*). The decreased incidence was not attributable to the reduced survival of the mice, since no excess mortality compared with controls was observed, even after a thoracic dose of 30 Gy. A recent paper from the same laboratory, using the same RFM mouse strain, stated that thoracic X-ray doses above 9 Gy could not be used because of animal lethality from lung damage within 180 days of exposure (*214*). The reasons for the differences between the experiments are not known.

Figure 10 shows some recent interesting dose-response data on adenoma induction in RFM mice, after thoracic X-rays and neutron exposure (*215*). The use of the parameter, tumor mouse, is based on the premise that the observed tumors are randomly distributed and that no sensitive or resistant subpopulations exist (*216*). Although these response relationships are probably the best available, they are nevertheless inadequate to define the precise shape of the dose-response curve for either radiation. The data are adequate to exclude both a linear dose response for X-rays and a square-root-of-the-dose relationship after neutron exposure. They strongly suggest a linear dose-squared relationship after X-irradiation and a linear response after neutron exposure. Such findings reinforce certain fundamental radiobiological theories of the interaction of radiation with matter and show that the relative biological effectiveness of neutrons increases with decreasing neutron dose (*217*).

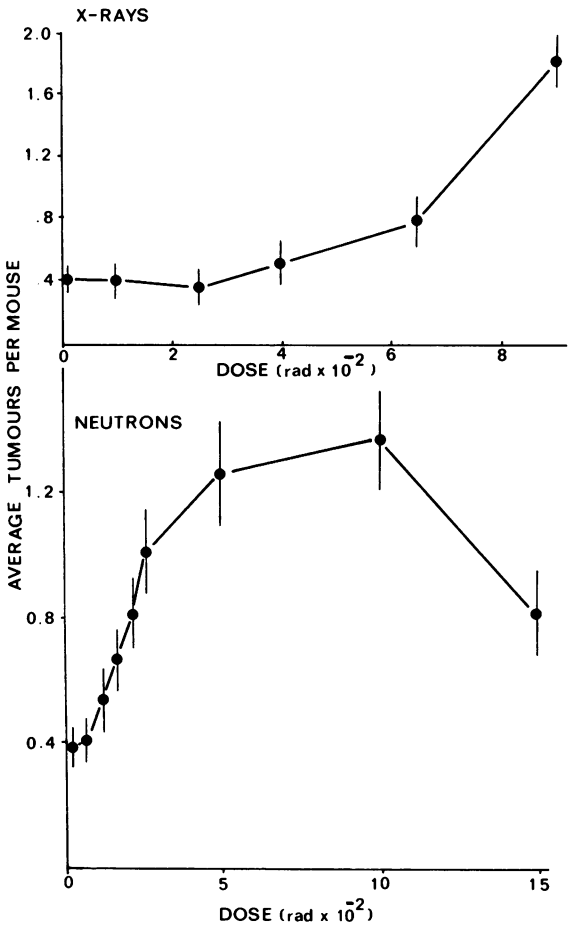

FIGURE 10. Relationship between lung tumors per mouse in female RFM mice, 9 months after localized thoracic exposure to X-rays (upper panel) and fission neutrons (lower panel). From R. L. Ullrich et al. (*215*), courtesy of the authors and Academic Press.

Nevertheless, even these data are ultimately limited because they involve a single sacrifice time and it will be necessary to do lifespan studies to resolve the basic questions as to whether the radiation truly induced tumors or merely accelerated their natural appearance.

Studies into the influence of dose rate (*214*) and of dose fractionation (*216*) reported that fewer lung tumors were induced by gamma-irradiation given at a low dose-rate (0.083 Gy per day) compared with a high one (0.45 Gy per minute). Although such studies are inherently difficult to interpret, these lung cancer data seem directly attributable to the differences in dose rate.

The fundamental radiobiological differences between the tumor dose response curves for X-rays and neutrons (see Fig. 10) were reinforced in split-dose studies (*216*). Thus after X-irradiation, recovery was observed using a 24 hr fractionation interval only when the total dose was on the dose-squared region of the single dose-response curve, i.e., above 4 Gy in Figure 10. In contrast, no recovery was observed using split doses of neutrons whatever the dose or whether the time interval between doses was 24 hr or 30 days. As Ullrich noted (*216*), these data imply that the primary mechanism of radiation carcinogenesis involves intracellular lesions and intracellular repair. It has nothing to do with intercellular interactions or repopulation phenomena.

Experimental Studies with Internal Irradiation

INTRODUCTION. The inhalation of radioactive material is a potentially important source of irradiation for radiation workers, especially those in the nuclear power industry. In power stations, the hazard is mainly from beta/gamma particles arising from the activation and corrosion of structural materials (*218*), whereas uranium and plutonium are more significant in the fabrication and reprocessing of reactor fuels.

Ideally our estimates of the hazard from radioactive particles should be based on human experience but, although follow-up studies are being carried out on men who have inhaled particulate plutonium, development of cancer as a result of exposure to actinides has not yet been documented. However, data on carcinogenesis from uranium miners who inhaled radon and its daugh-

ters have been reported in detail. Unfortunately, there is a considerable difference between the dose pattern produced by radon daughters and that produced by other radioactive particles that could lodge in the lung and give very high local doses. It is also proving difficult to determine the precise doses the miners received and to what extent their lung tumors were due to radiation alone. In the absence of direct human experience, we must turn to animals to improve our assessment of the lung cancer hazard from radioactive materials.

Neoplasia in the lung as a direct consequence of inhalation or instillation of radioactive materials is easily demonstrated in animals. It is often claimed that the most commonly used animals—rodents—are poor models for man, because both their spontaneous and radiation-induced tumors normally occur in the alveolar region, i.e., where the radiation dose is highest. In man, lung tumors tend to be of small-cell origin and located in the bronchiolar regions. The study of such neoplasia in animals and its extrapolation to man is also complicated by a number of other factors, not the least of which are the size of the inhaled particles, their chemical and physical form, the type of radioactive emission, the physical half-life of the material, and its biological half-life in the lung. Also, for most radioisotopes emitting alpha or beta particles, the dose delivered to the lung will be far from uniform. In addition, the inevitably protracted nature of the dose often makes comparisons with external X- or gamma-ray exposures inappropriate and even misleading. In terms of dose-effect relationships, because such parameters as deposition and the movement of particles are often unknown, it is difficult to establish a clear relationship between the physical features of contamination and the biological assessment of neoplasia. For instance, according to the characteristics of the deposit, the number of cells at risk varies. This underlines the difficulty in extrapolating from one species to another and of making direct comparisons between animals of different sizes. Nevertheless, in the absence of any evidence on the location of radiation-induced lung tumors in man, models based on data from rodents or dogs should be of relevance.

A useful discussion of the role of animal experiments is given in the UNSCEAR (1972) report on the effects of ionizing radiation (*219*). This concluded that there are two applications of general acceptability: (a) to help clarify the mechanisms of carcinogenesis and (b) to establish generalizations about the effects of radiation that apply to all species.

Experimental data are grouped here, and reviewed, according to the type of radioactive materials involved. The classification is one of relative "solubility," used in the sense of "transportable" within the body.

"SOLUBLE" ALPHA-EMITTERS. Lung cancer has been observed in several animal species after inhalation, or intratracheal injection, of relatively soluble (easily translocated) alpha-emitting radionuclides. Yuile and co-workers (*220*) observed adenocarcinomas and squamous cell carcinomas in equal numbers in rats after inhalation of ^{210}Po with a sodium chloride carrier. Deposition in the lungs giving doses in the range 0.71–5.83 Gy (after 280 days) resulted in cancer incidences of 4–13%. In Syrian golden hamsters given 15 weekly intratracheal injections of polonium absorbed on ferric oxide carrier particles, the incidence of bronchogenic carcinoma after 1 year was 91% at 45 Gy, and 43% at 2.25 Gy (*221,222*). In another experiment comparing the effects of ^{210}Po absorbed on Fe_2O_3 and ^{210}Po in saline, the authors concluded that a more uniformly distributed dose produced a higher cancer incidence (*223*). A comparison between lung cancers induced by ^{210}Po and by benzo(α)pyrene (*224*) emphasized the fact that the radioactive material induced peripherally sited cancers almost exclusively, whereas benzo(α)pyrene most frequently induced epidermoid carcinoma of the trachea or major bronchi.

Many lung tumors have been observed with higher actinides such as americium, curium, and einsteinium in the 3+ valence state. For example, both inhaled ^{241}Am nitrate and the relatively soluble oxide caused an increased incidence of lung carcinoma in rats (*225–227*). Both forms of americium resulted in a lung tumor incidence increasing with dose, as long as the rats' lifespans permitted cancer development, but the oxide seemed more likely to produce lung cancers than the nitrate. However, many other factors could have had an influence, e.g., rat strain and the appearance of many sarcomas in the americium oxide-exposed rats, giving a higher incidence than if only carcinomas had been recorded. As was observed with polonium and benzo(α)pyrene (*224*), the addition of another carcinogen, in this case, tobacco smoke, resulted in a 2- to 4-fold increase in cancer incidence.

Thorium administered by inhalation as $^{227}Th(NO_3)_4$ has been shown to give comparable results to inhalation of americium (*53*), whereas the incidence of lung tumors after inhalation of ^{244}Cm nitrate was not so high as might have been predicted. As curium considerably reduced the life span, it is possible that the full cancer potential was not expressed. Einsteinium has also been shown to induce lung cancers in rats after intratracheal instillation (*66,228*). Because of its relatively short half-life, it was necessary to inject high activities, which may have reduced the observed numbers of lung cancers. Compared with other transuranics, both curium and einsteinium cause high incidences of extrapulmonary tumors (*226,228*).

Various soluble forms of plutonium-239 have also been shown to increase lung tumor incidence, e.g., ^{239}Pu citrate and ammonium ^{239}Pu pentacarbonate inhaled by rats and rabbits (*229*), intratracheal injection and inhalation of ^{239}Pu nitrate and intratracheally injected ^{239}-plutyl acetate in rats (*230*). In addition, high incidences of lung tumors have been reported following inhalation of ^{238}Pu nitrate by rats (*231*).

In summary, relatively soluble alpha-emitters at IADs of greater than 0.04 MBq/kg have an increasing probability of causing lung cancer. However, the production of low tumor incidences at high doses reflects the shortened life spans due to deaths from causes other

than neoplasia. Below 0.04 MBq/kg no experiments have shown statistically significant increases in the lung tumor incidence. Several studies have provided evidence of a synergistic effect between alpha-radiation and tobacco smoke or benzo(α)pyrene.

"INSOLUBLE" ALPHA-EMITTERS. One of the first studies providing evidence that high insoluble alpha-emitting particles deposited in the lungs could cause cancer was that of Temple, Marks, and Bair (*232,233*) using intratracheal instillation of about 0.37 MBq/kg of $^{239}PuO_2$ into mice. In subsequent experiments, the exposure, this time by inhalation, was reduced to an IAD of 0.19 MBq/kg, but no cancer was induced (*234*). More recent studies with $^{239}PuO_2$ have been undertaken using hamsters and dogs. For example, whereas very little neoplasia was recorded in hamsters with IADs greater than 5.6 MBq/kg (*235*) in an experiment with dogs, 91% of the animals had tumors even at IADs of 0.26 MBq/kg (*236*). In this latter study, all the tumor-bearing animals had bronchioalveolar carcinomas with metastases to many other organs.

$^{238}PuO_2$ has also been used in a number of studies. Neoplasia was found in rats after inhalation of $^{238}PuO_2$ at IADs down to 0.14 MBq/kg lung (*237*), but no lung tumors were found in hamsters at IADs up to 20.4 MBq/kg lung (*238*). Several dog experiments are in progress, with neoplasia recorded at levels of about 1.1 MBq/kg lung, after inhalation of $^{238}PuO_2$ (*239,240*).

One of the most comprehensive neoplasia studies was the work reported by Sanders and co-workers (*241,242*), using high-fired $^{238}PuO_2$ and $^{239}PuO_2$ inhaled by rats. Oxides of plutonium exhibit varying degrees of solubility in the lung depending on their physicochemical form, but the particular method of preparation of the oxides in these experiments resulted in inhaled particles which, although differing considerably in specific activity, were not significantly different in *in vivo* solubility. The lung burdens chosen enabled complete dose-response curves to be obtained with peak incidences of lung tumors at about 20 Gy for ^{239}Pu and 100 Gy for ^{238}Pu, but with some increase detectable below 0.10 Gy (see Fig. 11). They also found significant relative differences in the types of tumors induced by the two isotopes: that is, many more adenocarcinomas were induced at low radiation doses after $^{239}PuO_2$ exposure than after $^{238}PuO_2$.

Attempts have also been made to investigate the effects of highly localized plutonium in the lung. The plutonium has been introduced as $^{238}PuO_2$ injected through the thoracic wall (*243*) in rats, or as ^{238}Pu microspheres by intubation, surgery or intravenous injection into the lungs of dogs (*236,243*), rats (*244*), and hamsters (*246*). All these experiments were largely unsuccessful in producing lung neoplasia.

The results from beagle dog experiments could be more relevant for extrapolation to man, although only the high dose groups are really complete; that is, doses in the range 10–120 Gy. These correspond to survival times between 2.5 and 11 years giving high incidences of tumors (82%) after $^{239}PuO_2$ (*236*). In a group of eight dogs receiving 0.26 MBq/kg, with an average life span of 8 years, the incidence was 87.5%. However, the small numbers involved and the apparent saturation of effect does not allow any insight into the dose-effect relationship. All pulmonary cancers observed in the beagles were adenocarcinomas although metastasis was frequent. There are also results from ^{238}Pu but again too few (two groups of ten dogs) to be of immediate use (*239*).

A few experiments have been started with primates (*247–249*) but, although the tumors induced have been similar to those occurring in man, insufficient numbers of animals have been used to make meaningful deductions.

Recent experiments (*250*) using a wide dose range, have involved the single inhalation exposure of mice to aerosols of sized $^{239}PuO_2$. Peak lung cancer incidence was 85% compared with a control value of 31%, following an initial lung deposition 92.5 Bq (0.6 MBq/kg). More significantly, a peak incidence of 52% was found compared with 10.5% in controls in mice killed 1 year after the same IAD. This would have been equivalent to a mean lung dose of 2.6 Gy (see Fig. 12).

There have also been a number of studies where repeated inhalation exposure of $^{239}PuO_2$ was used (*251–253*). These studies were prompted by the need to more closely match the expected intake in man. Lundgren's work with hamsters, which involved seven bimonthly exposures to ^{169}Yb-labeled $^{239}PuO_2$ particles (*252*), only produced one tumor. This emphasizes the low sensitivity of this species to the induction of lung neoplasia. However, from these data it was concluded that the incidence of lung tumors appeared to depend only on the cumulative radiation dose to the lung rather than the dose-rate; an observation confirmed recently in rats (*253*). This is in contrast to earlier work with mice, when protraction of the alpha dose by re-exposure caused a threefold increase in lung adenomas compared with a single exposure to the same lung burden (*251*).

In summary, it can be seen that relatively insoluble alpha-emitters produced neoplasia in the lungs at IADs above 0.04 MBq/kg in rats, mice, and dogs, with peak incidences in the range 0.6–3.7 MBq/kg. This range would be equivalent to mean lung doses of 2.5–12 Gy. Hamsters seem comparatively insensitive to the induction of lung tumors by all forms of alpha-emitters (*254–256*).

RADON AND RADON DAUGHTERS. Radon and its decay products present special problems in inhalation toxicology because, as discussed above, long-term effects have been recorded following human exposure in uranium mines (*257–260*). Experiments involving animal exposures have been used to develop a better basis for setting exposure standards. In particular, they have been used to determine whether other factors, such as cigarette smoke and ore dust, act synergistically with radiation in inducing neoplasia.

Pulmonary cancers have been observed in beagle dogs and hamsters that have inhaled radon decay products and ore dust (*261–263*), and in rats (*264*) inhaling radon. In this latter experiment exposures were protracted

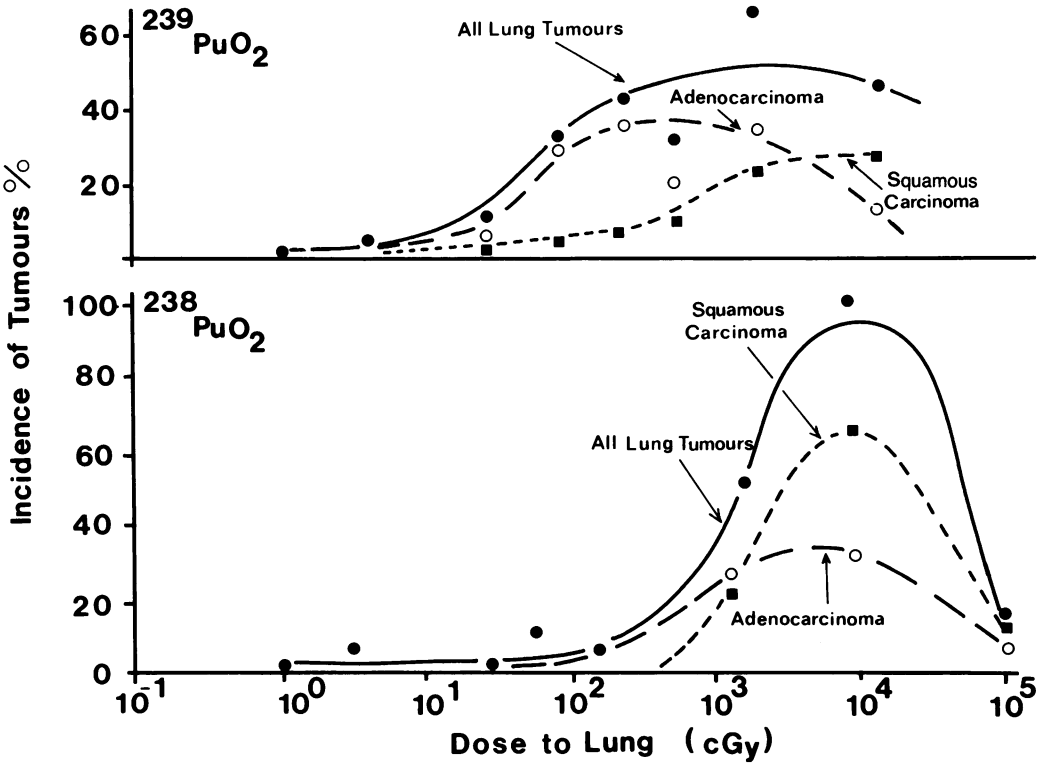

FIGURE 11. Comparison of lung tumor incidence data following inhalation of $^{238}PuO_2$ and $^{239}PuO_2$ by rats. From C. L. Sanders et al. (*242*), courtesy of the authors and Academic Press.

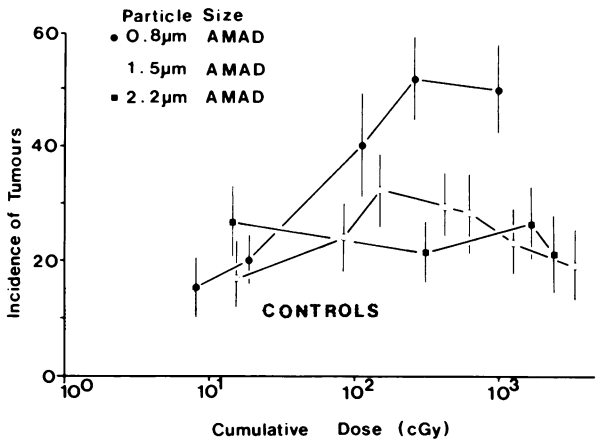

FIGURE 12. Incidence of lung tumors in mice, 1 year after inhalation of aerosols of sized $^{239}PuO_2$. (*250*).

and effects were related to working level months (WLM). The highest incidences of tumors (72 and 60%) were produced by exposures of 4500 and 9000 WLM respectively, delivered over 300–500 hr during a 3–4 month period (*264*). Over 14,000 WLM, the animals died of fibrosis before tumors could develop. Uranium dust seemed to have no effect on the incidence of tumors, which were about half bronchogenic carcinomas and half bronchioalveolar carcinomas. It appeared that a relatively high exposure, given over a few months early in life, was more likely to cause cancer than a protracted exposure throughout life. This was supported by Stuart and co-workers (*262,263*) in experiments with rodents and dogs chronically exposed to radon and uranium dust. Again, the tumors found in both rats and dogs were dissimilar to the oat-cell carcinoma commonly found in miners. In rats, the tumors were squamous carcinomas and adenocarcinomas, while in dogs, they were epidermoid and bronchioalveolar carcinomas, even though the local deposition in the lung was similar in each species. In these experiments, cigarette smoking did not appear to increase the frequency of cancers, but did promote other respiratory tract lesions, including pleural thickening, alveolar septal fibrosis, vesicular emphysema and chronic bronchitis. This action of smoke was again found in a recent comprehensive series of experiments by Cross and co-workers (*265*), involving beagle dogs inhaling radon daughters, uranium ore dust, and cigarette smoke. However, the carcinomas, which were of the epidermoid and bronchioalveolar type, appeared only in animals that had cumulative radon daughter exposure exceeding 13,000 WLM. This is nearly two orders of magnitude higher than that reported to cause lung cancer in man (*258*). Strangely, the tumor-bearing animals also survived longer than nontumor animals.

From all the data on the effects of radon, ore dust, and smoke (*266–270*) it is evident that although local conditions in miner's respiratory tracts can be simulated

in animals, the effects have been different with the results applicable in only a general manner, i.e., in terms of modeling. Nevertheless, there is evidence at least in rats that the carcinogenic actions of alpha radiation and either tobacco smoke or benzo(α)pyrene are synergistic, but are highly dependent on the mode of administration of the cocarcinogen.

BETA/GAMMA-EMITTING SOURCES. In attempts to produce intense local fields of radiation in the lung, several experiments have reported the use of ^{90}Sr (*271,272*) or ^{106}Ru (*273–275*) in the form of labeled thoracically implanted spheres or rods. In the study of Cember and Watson (*271*), 7 out of 23 rats died of lung tumors with doses of $0.5–2.6 \times 10^3$ Gy from ^{90}Sr-labeled glass beads in the lung. In all these experiments, considerable trauma was associated with the implantation of the source and even with ^{106}Ru no tumors were seen below 3.2×10^3 Gy, making comparisons with inhaled materials difficult. Nevertheless, this procedure was utilized in one study, in which ^{60}Co was used to examine interspecies differences in lung tumor incidences—lowest incidence was in hamsters (8%) and the highest in rats (75%) (*276*).

Intratracheal instillation allows a fairly accurate assessment to be made of the amount of material given to the animals, but produces more trauma and a more uneven distribution of the material than inhalation (*277*). Rats have been used almost exclusively for these experiments, for example, using $Ba^{35}SO_4$ (*271,278*), $^{144}CeF_3$ (*279,280*) and $^{144}CeCl_3$ (*281*). The lung tumors seen have been squamous cell carcinomas or bronchogenic ones with little apparent dependence on the initiating isotope, but with lung burdens about three orders of magnitude higher than for alpha-emitting materials.

Most experiments with beta-emitters have involved the inhalation of various compounds of ^{144}Ce, either in rats (*282,283*) or mice (*284*). These experiments have involved few animals and produced few tumors, especially in mice. There have also been a number of dog experiments using ^{90}Y, ^{91}Y, ^{144}Ce, or ^{90}Sr in fused aluminosilicate particles (*285*). The responses in these studies were enough to allow comparison with the inhalation of $^{239}PuO_2$ by dogs (*236*). Hemangiosarcomas were induced in animals that had been exposed to ^{144}Ce and ^{90}Sr but were not found following ^{90}Y, ^{91}Y, or $^{239}PuO_2$ exposures. $^{239}PuO_2$ experiments were characterized by the predominance of bronchioalveolar carcinomas.

THE "HOT PARTICLE" THEORY. Little is known about the movement of individual particles in the lung of man, but insoluble material may be retained within the pulmonary region for hundreds of days. Even in the tracheobronchial region, from which most material is very rapidly cleared, there is evidence that some particles are not moved for hours or days (*286,287*). In the case of particles emitting radiation of a limited range, it is therefore possible that very high doses may be received by the small volumes of tissue surrounding such particles, whilst most of the organ remains virtually unirradiated. This would suggest that the effects of such inhomogeneous radiation would not relate to the "averaged" dose over the whole lung. There has been considerable controversy in the literature about the magnitude of long-term risks associated with exposures of this type (*288*). This "hot particle" problem is an extreme example of the more general problems of assessing the consequences of nonuniform irradiation. It is not a new concept and the International Commission on Radiological Protection (ICRP) considered it in 1969 (*289*). The current dose limits are based on the assumption that using mean organ dose will not underestimate the risks, although the limitations are appreciated.

Most data indicate that homogeneous dose distributions are more effective, although Sanders, Thompson, and Bair (*290*) in reviewing the available evidence from animal experiments concluded that nonuniform internal irradiation of the lung was more carcinogenic than uniform exposure for the same amount of absorbed energy. The suggestions by Tamplin and Cochran in 1974 (*288*) that the risks from "hot particles" may be several orders of magnitude higher than would be expected on the basis of mean organ dose, came not from direct observation but were the predictions of hypotheses concerning the mechanisms of radiation carcinogenesis (*291,292*).

Geesaman (*291*) suggested that there were small critical volumes of tissue which, if given a dose sufficient to disrupt them had a probability of producing a tumor of the order of 10^{-3} to 10^{-4}. This hypothesis was based on the results of an experiment in which rat skin was irradiated (*293*) and which showed an apparent correlation between the number of atrophied hair follicles and the number of tumors produced. Dean and Langham (*292*) proposed a model, which assumed that tumors arose from a single irradiated cell. They obtained a cellular dose-response relationship from the result of an experiment in which rat skin was uniformly irradiated (*294*), by assuming that a single layer of cells—the basal layer—was at risk. By applying this dose-response relationship to all cells in the lung they found a very high probability of tumor induction from a single radioactive particle.

Both models assumed that all lung tissue was as radiosensitive as certain structures within the skin, which were taken to be unusually susceptible to tumor induction. This hypothesis was vigorously supported by Tamplin and Cochran (*288*), but equally vigorously criticized by others (*295–298*). Most of these criticisms were as theoretical as the original hypothesis, but there have since been a number of experiments which are pertinent. For example, Anderson and co-workers (*246*), using plutonium-containing particles deposited in the pulmonary capillaries of hamsters, showed no enhancement effect of particulate compared with more uniformly distributed plutonium. This work, although producing few tumors, has been extended to other species with the same result (*299–302*). In these experiments no pulmonary neoplasia was recorded until more than 36% of the lung was irradiated with a large dose rate (130 Gy/yr) and then with only 10% incidence. It is possible that

this lack of neoplastic effect is related to the lack of movement of the irradiating particles, which lodged in the pulmonary capillaries. If this were the case, the number of irradiated cells becomes an important factor in the carcinogenic effects. Experiments involving inhalation of finely crushed $^{238}PuO_2$ (*237,303*), which delivers a particularly uniform dose to the lung, also support the theory of increased effect for more uniform irradiation. In more recent studies in our own laboratories (*250*), long-term effects were recorded in mice that had inhaled aerosols of sized $^{239}PuO_2$. The aerosols had AMADs of 0.8 μm, 1.5 μm, and 2.2 μm. In this way, different dose distributions in the lung would be produced, as different numbers of particles were inhaled for the same IAD. Some of these mice were killed at one year and others left for their lifespan. In both groups, the maximum incidence of lung tumors occurred following the most uniform distribution of dose, i.e., after inhalation of the smallest size particles. This provides sound experimental evidence that uniform irradiation of the lung is more hazardous for long-term effects than a highly nonuniform dose distribution.

CONCLUSION. Evidence from animal experiments on radiation-induced lung cancer has been reviewed at various times over the last 20 years or so (*53,290,304–306*). Other reviews (*158,297*) have concentrated on comparing the toxicity of alpha radiation from insoluble plutonium particles with that from uniformly distributed alpha activity in the lung.

From the reviewed data certain general conclusions may be made. The speed of development of tumors, i.e., the latent period, appears to be proportional to the dose, although a series of well-defined morphological changes always occur even in control animals. If the lifespan of rats with pulmonary cancer is taken into consideration, a clear difference of about a factor of three in terms of risk is apparent, between animals exposed to relatively transportable elements such as ^{241}Am and nontransportable materials such as ^{239}Pu oxide. These differences are most marked at low doses but interpretations are complicated by factors connected with experimental design.

There is now a body of animal evidence that suggests that the carcinogenic action of alpha radiation and other agents such as tobacco smoke and benzo(α)pyrene in the lung may be more than additive. However, these effects seem highly species-specific and dependent on the mode of administration of the two agents.

In rodents, radiation-induced cancers can be divided into three types according to the cells in which the tumors originate: bronchioalveolar carcinomas (type II, pneumonocytes); bronchogenic carcinomas (epidermoid); and sarcomas.

Sarcomas represent only about 2% of cancers, the remainder being equally bronchioalveolar and bronchogenic carcinomas (*225*). At low doses, bronchioalveolar cancers predominate, while at high doses it is bronchogenic cancers that assume this role. This may reflect the different speeds of development of the two types of tumor. A similar distribution of tumor type is also found after inhalation of radon and its daughters whose mode of deposition is quite different. This illustrates the importance of cell-type sensitivity rather than the region where most damage occurs.

The combined dog, rat, and mouse survival data more closely correlate with average dose per day than with total dose, suggesting that repair mechanisms enhance survival at lower dose rates.

The peripheral areas of the lung, which seem to concentrate inhaled $^{239}PuO_2$, are the sites where $^{239}PuO_2$-induced tumors occur in rats and mice. Thus, it might be concluded that peripheral tumors would be the type induced by plutonium in man, in contrast to the more central or hilar location of cancer in cigarette smokers (*307*). Peripheral lung tumors are also the most common spontaneous type occurring in dogs (*308,309*), in which the radiation exposure may be merely increasing their incidence. It has also been suggested that peripheral tumors could be the type most prevalent in man if tumors resulting from smoking-related causes were removed from consideration (*310*).

Even with this evidence, the critical steps in the development of radioactive particle-induced lung tumors remain unknown. After inhalation, particles are phagocytosed by alveolar macrophages, and there are theories that neoplasia is a consequence of cell death, tissue necrosis and fibrosis (*311*). This mechanism is supported by the incidence of fibrosis-linked tumors in man (*312*).

In summary, it is clear that although animal experiments have provided a mass of data, the elucidation of the mechanism of lung carcinogenesis is far from complete. This is partly because of the complexicity of the subject and partly because of the often overriding desire to simulate practical conditions. However, certain objectives have been reached: it can be seen that, in terms of carcinogenic risk, alpha-emitting material is at least an order of magnitude more hazardous than that emitting beta/gamma radiation. Also there is considerable evidence that a more homogeneous irradiation of the lung produces more tumors than "hot spot" dose patterns. Finally, it is still unfortunately the case that we do not yet know which species can provide the best model for extrapolation to man.

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